DOCTOR OF MEDICINE

The Acute Effects of Allopurinol in Angina

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Degree of Doctor of Medicine

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Abbreviations

ACC	American College of Cardiology
ACE	Angiotensin converting inhibitor
ACS	Acute coronary syndrome
AMP	Adenylic acid
ATP	Adenosine triphosphate
BNP	B-type natriuretic peptide
BCS	British Cardiovascular society
CABG	Coronary artery bypass graft
CAD	Coronary artery disease
CSC	Canadian Cardiovascular Society
CHD	Coronary heart disease
CVD	Cardiovascular disease
CRP	C Reactive protein
ECG	Electrocardiography
ECS	European Society of Cardiology

ETT	Exercise tolerance test
GTN	Glycerotrinitrate
GMP	Guanylic acid
IMP	Inosinic acid
LDL	Low density lipoprotein
LIFE	Losartan Intervention for Endpoint Study
MPS	Myocardial Perfusion Scan
MMPs	Metalloproteinases
NADPH	Nicontinamide adenine dinucleaotide phosphate
NHANES	National Health and Nutrition Examination Survey
NO	Nitric oxide
NSTEMI	Non ST elevation myocardial infarction
OS	Oxidative stress
PCI	Percutaneous coronary intervention
ROS	Reactive oxygen species
STEMI	ST elevation myocardial infarction

SUA	Serum uric acid
XD	Xanthine dehydrogenase
XO	Xanthine oxidase
XOR	Xanthine oxidase reductase

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Declaration

I hereby declare that I am the author of this thesis, that all references cited have been consulted by me and that I have carried out the work described within.

The work described in this thesis has not been previously accepted for a higher degree and I have defined the nature of my contribution to the work within the project described in the thesis.

The work contained in this thesis was carried out during my appointment as a Clinical Research Fellow in the Centre for Cardiovascular and Lung Biology, Division of Medical Sciences, Ninewells Hospital & Medical School, University of Dundee, between March 2012 and July 2014.

Signed_____ Date_____

Summary

Angina is pain or constricting discomfort that typically occurs at the front of the chest and may radiate to neck, shoulders, jaw or arms and is precipitated by physical exertion or emotional stress which increases myocardial demand. Allopurinol has been shown to prolong the time to ST depression on ETT, which is a marker of ischaemia. This may be via a number of mechanisms including effect on oxidative stress and endothelial stability.

This three arm cross over, double blind study was designed to establish the time of onset of effect of allopurinol and the optimal dose. The primary outcome was time to ST depression. Secondary outcomes were total exercise time and effect on troponin, oxidised LDL, CRP and BNP levels.

There were 26 participants with a mean age of 70.1 and 77% male. 5 participants did not complete the study.

Due to challenges of recruitment this study was underpowered but it did appear at 24 hours post study drug administration there was an impact on both time to ST depression and total exercise time in the active arms of the trial. Furthermore it was shown total exercise time improved irrespective of whether the participant was in an active or

placebo arm. This may suggest the protocol was contaminated by the training effect of repeated high intensity exercise.

1 Introduction

1.1 Angina

1.1.1 Definition of angina

Angina is pain or constricting discomfort that typically occurs at the front of the chest and may radiate to neck, shoulders, jaw or arms and is precipitated by physical exertion or emotional stress which increases myocardial demand.[1-4] Classic stable angina is predictable in onset, reproducible and relieved by rest or glycerol trinitrate (GTN). Angina is the main symptom of myocardial ischaemia and is usually caused by atherosclerotic obstructive coronary artery disease (CAD) restricting blood flow and therefore oxygen delivery to the heart.

Angina can be graded by severity on the Canadian Cardiovascular Society (CCS) class scale:[2]

Class I	Ordinary activity such as walking or climbing stairs does not precipitate angina
Class II	Angina precipitated by emotion, cold weather or meals and by walking upstairs
Class III	Marked limitations of ordinary physical activity

Class IV Inability to carry out any physical activity without discomfort – anginal symptoms may be present at rest.

The likelihood of diagnosis of angina increases with the number of cardiovascular risk factors present. These include smoking, hypertension, diabetes, family history (first degree relative aged < 65 years), and hypercholesterolemia.

1.1.2 Epidemiology

The Health Survey for England (2006) reported around 8% of men and 3% of women between 55 and 64 years currently have or have had angina.[1] The figures for men and women aged between 65 and 74 years are around 14% and 8% respectively .[1] The Scottish Health Survey (2003) reported similar prevalence with 5.1% and 6.7% in males aged 55-64 and 65-74 respectively. For the same age groups in woman the rates were 4% and 6.8%.[2]

1.1.3 Diagnosis of angina

A baseline 12 lead electrocardiograph (ECG) should be performed in every patient suspected of having angina. The majority of patients will then undergo an exercise tolerance test (ETT). The sensitivity and specificity of this test depends on the cohort of patients studied. Sensitivity is higher in patients with triple vessel disease and lower in

those with single vessel disease. [3, 5] The true diagnostic value of ETT lies in its relatively high sensitivity but it is only moderately specific for diagnosis of CAD in women.[3, 6]

Myocardial perfusion scintigraphy (MPS) with exercise or pharmacological stress is an alternative accurate and non-invasive investigation which can predict the presence of CAD. It has a useful role in those patients unable to exercise adequately on ETT or with ECG abnormalities which make ETTs difficult to interpret. It is also useful in females who may have low risk of underlying CAD but a high risk of falsely positive ETT and in patients in whom identification of regional ischaemia would be of value (i.e. prior to percutaneous coronary intervention(PCI))

Coronary angiography is the benchmark investigation for establishing nature, anatomy and severity of CAD. In patients with stable angina it should only be considered if patients are identified as high risk or if diagnostic uncertainty remains.[2]

NICE have issued guidelines on the approach to investigating patients presenting with stable chest pain based on the likelihood they will have CAD and advise 'if people have features of typical angina based on clinical assessment and their estimated likelihood of CAD is greater

than 90% (see table 1), further diagnostic investigation is unnecessary.

Manage as angina'.[7]

	Non-anginal chest pain					**Atypical angina**					**Typical Angina**			
	Men		Women			Men		Women			Men		Women	
Age in years	Lo	Hi	Lo	Hi		Lo	Hi	Lo	Hi		Lo	Hi	Lo	Hi
35	3	35	1	19		8	59	2	39		30	88	10	78
45	9	47	2	22		21	70	5	43		51	92	20	79
55	23	59	4	25		45	79	10	47		80	95	38	82
65	49	69	9	29		71	86	20	51		93	97	56	84

Table 1 Percentage of people estimated to have coronary artery disease according to typicality of symptoms, age, sex and risk factors[7]

1.1.4 Standard treatment of angina

Patients with stable angina should be prescribed a short acting nitrate and advised to use this prior to activities which provoke their angina.[1] Beta-blockers should be given as first line anti-anginal therapy and if patients cannot tolerate beta-blockers, consider calcium channel blockers. If symptoms remains poorly controlled consider a combination of both. For patients on beta-blocker or calcium channel blocker monotherapy whose symptoms are not controlled and who are intolerant of the other consider adding a long-acting nitrate, ivabridine, nicorandil or ranolazine. If patients cannot tolerate either beta-blocker or calcium channel blockers one of these can be given as monotherapy.

In addition aspirin, angiotensin converting enzyme (ACE) inhibitor, statin and treatment of elevated blood pressure should be given as secondary prevention.

Patients with poorly controlled symptoms despite optimal medical therapy should be considered for revascularisation with either PCI or coronary artery bypass graft (CABG).

1.1.5 Pathophysiology of angina

Myocardial ischaemia is due to an imbalance between myocardial oxygen supply and myocardial oxygen consumption (See figure 1).

Myocardial oxygen supply is determined by arterial oxygen saturation and myocardial oxygen extraction which are relatively fixed under normal circumstances and coronary flow which is dependent on the luminal cross sectional area of the coronary artery and coronary arterial tone. Coronary flow is also influenced by collateral blood flow, perfusion pressure which is determined by pressure gradients from the aorta to the coronary artery and, since flow is from epicardium to endocardium, from the coronary artery to the endocardial capillaries. The flow within the endocardium is determined by the left ventricle end diastolic pressure. In addition coronary flow is affected by heart rate which affects the duration of diastole; importantly coronary flow occurs primarily during diastole.[8]

Myocardial demand is determined by four major factors namely heart rate, systolic blood pressure, myocardial wall tension and myocardial contractility.

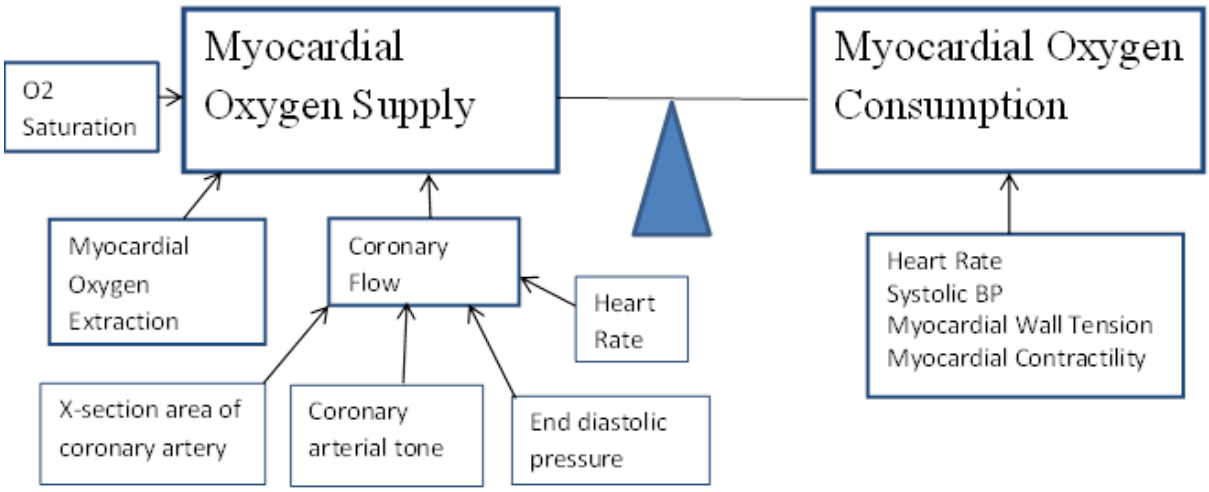


Figure 1 Diagram of components of myocardial supply and demand

The luminal cross sectional area of coronary arteries and the coronary arterial tone may be dramatically altered by the presence of atherosclerotic plaque in the vessel wall resulting in an imbalance of oxygen supply and demand when oxygen demand increases such as during exertion. The increase in demand is due to an increase in heart rate, myocardial contractility and wall stress.[3] Ischaemia induced sympathetic activation can further increase the severity of ischaemia through a variety of mechanisms which can result in further increase of myocardial oxygen consumption and coronary vasoconstriction. The term ischaemic cascade describes a sequence of events beginning with an imbalance between oxygen supply and demand, resulting in

metabolic abnormalities, perfusion mismatch and global diastolic and systolic dysfunction, ECG changes and often angina.[3 9]

In the majority of patients, the pathological substrate of stable angina is atheromatous narrowing of the coronary arteries. The normal vascular bed has the capacity to reduce resistance, allowing coronary flow to increase 5-6 fold during maximal exercise. Reduction in the luminal cross sectional area by atherosclerotic plaque reduces the normal ability of the coronary vascular bed to reduce its resistance during maximal exercise resulting in ischaemia. A luminal reduction of ≥ 50% may be associated with ischaemia because coronary blood flow becomes inadequate to meet demand during exercise or stress.[3] In addition, stenosis resistance changes relatively little with mild degrees of vascular narrowing but rises precipitously with severe obstruction such that resistance almost triples between stenosis of 80 and 90%. The ischaemic threshold is also influenced by other factors including the development of collateral circulation, the degree of transmural distribution of myocardial perfusion from the more vulnerable subendocardium to the subepicardium, coronary vascular tone and platelet aggregation.

Atherosclerosis is a chronic disease characterised by two fundamental hallmarks: lipid accumulation and inflammation. It is a progressive disease in which lipids, extracellular matrix and activated vascular smooth muscle cells accumulate in the arterial wall resulting in growth of atherosclerotic plaque. After the formation of fatty streaks, the atheroma typically evolves into a more fibrotic and complex lesion which eventually leads to the clinical manifestation of CAD. Endothelial dysfunction is also considered an important prognostic factor indeed a precursor to the development of atherosclerosis. [10]

The mechanisms responsible for the sensation of angina are complex and not fully understood. Ischaemia does not always manifest as pain and can be silent with painless ischaemia described in all coronary syndromes including stable angina, unstable angina, vasospastic angina and myocardial infarction.[3, 11] In addition the development of ECG changes may precede or follow the development of symptoms.[11]

Myocardial ischaemia reduces the formation of adenosine triphosphate (ATP) resulting in the development of acidosis, the loss of the normal ATP-sodium potassium pump, the loss of myocardial membrane integrity and the release of chemical substances that stimulate chemosensitive and mechanoreceptive receptors innervated by the

unmyelinated nerve cells found within cardiac muscle fibres and around the coronary vessel. The substances released include lactate, serotonin, bradykinin, histamine, reactive oxygen species and adenosine. [8, 12] In addition there are substances released from platelets which often spontaneously aggregate in the area of coronary artery stenosis which may also be responsible for myocardial ischaemia and angina. These include serotonin, thromboxane A2 and 5 hydroxytriptamine.[8 13]

There is substantial evidence the primary mediator of angina pain is adenosine via stimulation of A1 adenosine receptors.[8, 11, 14] It is also possible that venodilation, as a response to ischaemia, can active these receptors. The nerve fibres travel along the sympathetic afferent pathways from the heart and enter the sympathetic ganglia in lower cervical and upper thoracic spinal cord. Impulses are then transmitted via the ascending spinothoracic pathways to the medial and lateral thalamus and ultimately active several areas of the cerebral cortex resulting in the sensation of angina.

1.2 Acute coronary syndromes

Acute coronary syndromes (ACS) encompass a spectrum of disease from unstable angina to transmural myocardial infarction. All have the common aetiology in the formation of thrombus on an inflamed and complicated atheromatous plaque due to plaque erosion and rupture. The definition of ACS requires a triad of clinical presentation, ECG changes and biochemical cardiac markers. An ACS may occur in the absence of ECG changes or elevations in biochemical markers when the diagnosis is supported by presence of prior documented CAD or subsequent confirmatory investigations. [15]

1.2.1 Treatment of ACS

Immediate treatment is defined by the characteristics of the presenting ECG, in particular the presence of ST elevation. In combination with clinical presentation, ST elevation of ≥ 2mm in at least 2 adjacent precordial leads, ≥ 1mm in at least 2 adjacent limbs leads or the development of new left bundle branch block (LBBB) defines an ST elevation myocardial infarction (STEMI) and this is treated with emergency reperfusion. In the absence of ST elevation i.e. non ST

elevation ACS (NSTEMI) patients are initially managed without emergency reperfusion.

Unstable angina is differentiated from NSTEMI by the serum concentration of cardiac markers. The cardiac markers troponin I and troponin T are highly sensitive to myocardial damage. The European Society of Cardiology (ECS) and American College of Cardiology (ACC) state that any elevation of troponin or creatinine kinase MB (muscle, brain) should be classified as a myocardial infarction. The British Cardiac Society (BCS) and World Health Organisation (WHO) state those with no cardiac enzyme release should be classified as unstable angina, those with a minor troponin as ACS with myocyte necrosis and those with significant release as ACS with myocardial infarction.

First line treatment essential for all patients presenting with ACS should include aspirin and synthetic pentasaccharides (e.g. fondaparinex) or low molecular weight heparin. Subsequently patients should be commenced on beta blocker, ACE inhibitor and statin.

1.2.2 Pathophysiology of ACS

The pathophysiology of ACS is due to a fissure, erosion or rupture of an atherosclerotic coronary plaque. The loss of integrity of the protective covering over an atherosclerotic plaque exposes the highly

thrombogenic contents of the core of the plaque resulting in platelet aggregation leading to subtotal or total vessel occlusion. Activated platelets release a number of vasoconstrictors which may further impair coronary flow through the stimulation of vascular smooth muscle cells both locally and distally. The haemodynamic compromise of the atherosclerotic plaque prior to rupture may have been mild[16] and plaques are lipid filled with foam cells. Activation of inflammatory cells within the atherosclerotic plaque appears to play an important role in the destabilisation process. This is covered in more detail later on.

1.3 Uric Acid

1.3.1 Metabolism of Uric Acid

Uric acid (UA) is the end of product of the metabolism of purine compounds, produced in the liver from the degradation of dietary and endogenously synthesised purine compounds. Purine mononucleotides, guanylic acid (GMP), inosinic acid (IMP) and adenylic acid (AMP) are broken down into purine bases, guanine and hypoxanthine. These are then metabolised to xanthine and subsequently uric acid by the action of xanthine oxidase (XO). (See figure 2 and 3)

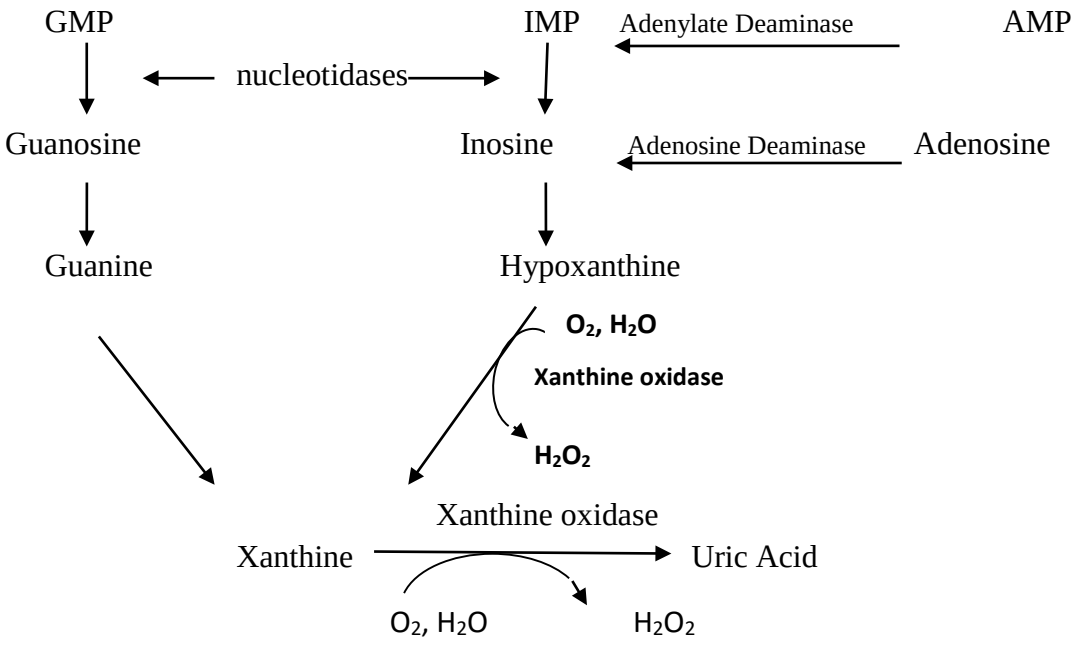


Figure 2 Metabolism of uric acid

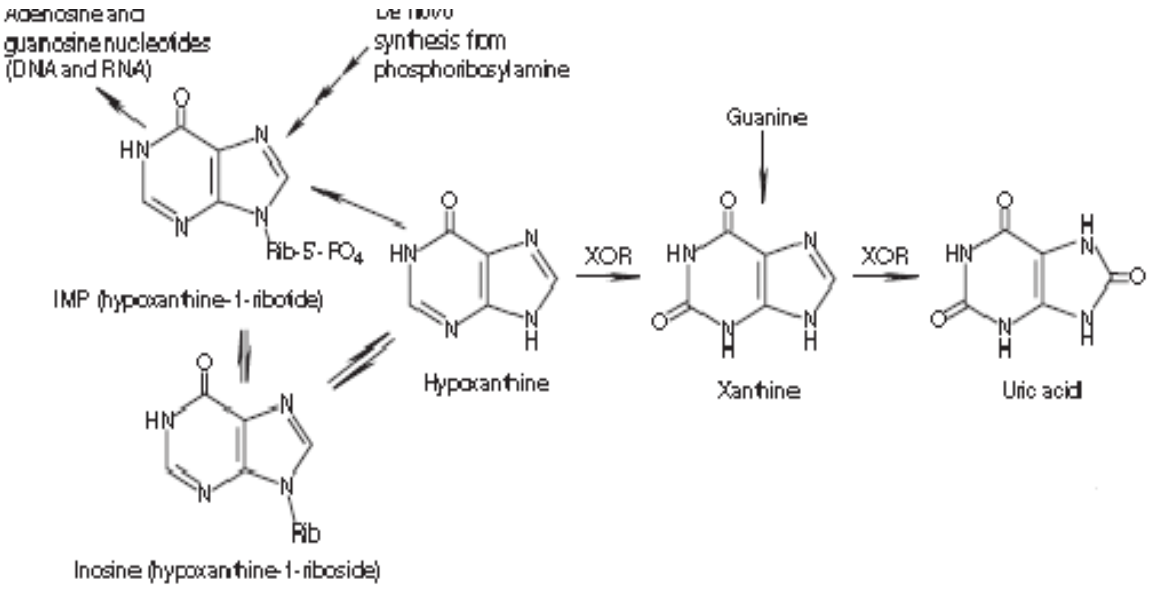


Figure 3 Structure of uric acid[17]

Xanthine oxidoreductase (XOR) exists as two interchangeable forms, XO and xanthine dehydrogenase (XD). The term XOR is used to describe both forms of the enzyme. XOR is part of a group of enzymes known as the molybdenum iron-sulfur flavin hydroxylases. XOR is widely distributed throughout various organs including the liver, intestine, lung, kidney, heart, brain and plasma with the highest levels being found in the small intestine and the liver.[18-20] In the myocardium, it is largely localized to the capillary endothelial cells, although the vascular

endothelium and less so the myocardial cells contain xanthine dehydrogenase.

XD is converted to XO by limited proteinolysis or oxidation of about eight cysteine residues to cysteine. Both forms of XOR oxidise xanthines however XO directly reduces oxygen to superoxide. The superoxide anions then form hydrogen perioxide and hydroxyl radicals by interacting with hydrogen ions. These molecules are highly reactive and referred to as reactive oxygen species (ROS).

Uric acid is primarily secreted by the kidneys where it is completely filtered at the glomerulus and reabsorbed in the proximal tubule. Elevated serum uric acid may be the result of overproduction but is usually due to reduced excretion[21].

1.3.2 Uric acid and cardiovascular risk

Epidemiological links between elevated serum uric acid and increased cardiovascular risk has been recognised for many years. [22, 23] Many studies have suggested the serum uric acid is a risk factor for cardiovascular events[24-27] There is clear evidence from several studies that elevated serum uric acid is a strong independent predictor of hypertension and this was confirmed by a recent meta-analysis.[28] However although this relationship would implicate uric acid as an

indirect risk factor for cardiovascular disease, the question remains as to whether uric acid is a causal CV risk or something which increases concurrently with other causal factors, causing the CV risk. Many studies have suggested the former is the case…….[24-26, 29, 30] whereas others suggest the latter. [31] [32-36]

The National Health and Nutrition Examination Survey (NHANES) I Epidemiological Follow up Study[24] looked at almost 6000 patients aged 24-74 years with no previous myocardial infarction or stroke and the mean follow up was 16.4 years. Baseline serum uric acid (SUA) was available for each subject with the mean (SD) SUA 327 (86) µmol/l (range 42-744 µmol/l). Men had significantly higher SUA levels than women and black people significantly higher than white. Mean levels increased with age.

Patients were analysed in 4 quartiles; 1 – SUA less than 321 µmol/l, 2- SUA 321-263 µmol/l, 3- SUA 364-416 µmol/l and 4- SUA higher than 416 µmol/l . Those in the higher quartiles had increasing blood pressures, cholesterol levels and BMI. SUA levels were substantially higher in those reporting recent diuretic use or alcohol use. During the follow up period 1593 (26.9%) died with 731 (45.9%) of these deaths attributed to cardiovascular death. Compared with the lowest quartile, age and

race adjusted death rates were higher in quartile 4 for both men and women. This difference was significant in women.

Further analysis, controlling for other recognised risk factors, found the risk of cardiovascular (CVD) death increased as SUA levels increased in all groups except men using diuretics.

A further epidemiological study [25]looked at serum uric acid in patients with proven CAD on angiography. Almost 9000 patients were included in the analysis with baseline SUA levels and SUA level at 6 months documented. Patients were subdivided into 4 quartiles, < 4.7mg/dl, 4.8-5.7mg/dl, 5.8-6.7mg/dl and >6.8mg/dl and the incidence of all events and cardiovascular events were noted over a 3 year follow up period. The incidence of cardiovascular and cerebrovascular events and all-cause mortality were higher in the highest quartile than the others.

The Losartan Intervention for Endpoint reduction (LIFE)[29] study demonstrated the superiority of a losartan based regime over an atenolol based regime for reduction of cardiovascular morbidity and mortality. Sub analysis was performed to assess effect of baseline SUA on cardiovascular endpoints using a COX regression model. As has been previously report, baseline SUA was higher in men. After adjusting for other risk factors the analysis found baseline SUA remained significantly

associated with increased risk in women only. The distribution of patients on diuretics was equal in both groups.

A further epidemiological study assessed role of SUA in predicting mortality in type 2 diabetics.[26] 2845 patients with diabetes were included and followed up for 4.7 ± 0.8years (Range 1-5 years). Baseline SUA levels were noted with hyperuricaemia documented as ≥ 416 µmol/l in men and ≥ 386 µmol/l in women. During the follow up period 329 (12.1%) participants died with 145 (44.1%) dying from cardiovascular causes. After adjusting for several baseline factors, an independent association was found between serum uric acid levels and CVD mortality. This was not true however for all-cause mortality.

Another cohort study performed in Finland[30] included 1423 men with no prior history of diabetes, CVD or cancer. The main outcome was death from cardiovascular disease, with the mean follow up 11.9 years. There were 157 deaths during follow up with 55 of these CVD deaths. CVD mortality was associated with increased baseline SUA. Extensive adjustment for variables did not attenuate this association.

The Rotterdam study [37] was another large prospective, population based study which looked at 4385 patients aged over 55 with no prior history of stroke or coronary artery disease. Patients were divided into

4 quartiles based on their baseline SUA. The mean follow up was 8.4 years. High SUA was associated with risk of CAD. After adjustment for potential confounding factors this only slightly attenuated the association. The associated was noted to be stronger in patients with no hypertension. Patients on SUA influencing medication at baseline were excluded from analysis.

The Framingham Heart study [31]however was a large prospective observational study which followed 6763 patients for a mean of 23 years – the longest follow up period to date. The analysis was sex specific as men of all ages have a higher SUA than comparable women. Analysis of their data showed serum uric acid was not associated with increased risk for CHD, deaths from cardiovascular disease, or death from all causes. Of note, in the subgroup of patients on diuretics, baseline SUA was not associated with subsequent CAD, death from CVD or all-cause mortality. It has been noted the Framingham population was almost exclusively white whereas NHANES population was more heterogeneous.

Similar results were achieved in the ARIC study [32]another prospective population based study which followed a total of 13504 healthy middle aged patients for up to 8 years. Patients were divided into quartiles

based on their baseline SUA with quartiles divided as SUA <4.4mg/dl, 4.5-5.2mg/dl, 5.3-6.2mg/dl and >6.3mg/dl in women and <5.7, 5.8-6.5,, 6.6-7.5mg/dl and >7.6mg/dl in men. SUA levels were found to be directly associated with most risk factors for cardiovascular disease including hypertension, LDL cholesterol, triglycerides, body mass index and waist/hip ratio. After adjustment for other risk factors the relationship between SUA and SAD became non-significant. Excluding patients on diuretics and analysing the data resulted in conclusions identical to the original data.

A further cohort study[33] performed in 22696 men with abnormal cholesterol, blood pressure, fasting blood glucose or urinary protein on routine examination, followed the patients up for an average of 6.5 years (SD 1.9 years). The patients were divided into 4 quartiles of baseline SUA. Higher SUA was associated with hypertension, high cholesterol and obesity. During the follow up period there were 1625 all cause deaths, 387 cancer deaths and 323 atherosclerotic CVD deaths. The baseline uric acid level was not associated with increased risk of death in any quartile. In those with diabetes, SUA was associated with

increased risk of death from all causes, even after adjustment of the covariates.

The 1967-1973 Chicago Heart Association Dectection Project in Industry[36] screened 39665 employees of which 24997 had complete baseline data. The relationship between uric acid and 5-year mortality from all causes, from CVD and CAD, was analysed in patients free from CVD at baseline. Analysis of the 5 year mortality in white men age 45-64, in highest uric acid group, found all-cause mortality was higher. CVD and CHD death rates were also higher in men in the highest uric acid groups, even when hypertensives on treatment were excluded although the tendency was less strong. However when controlled for differences in other variables there was no significance between uric acid and CHD and CVD deaths. Analysis of 13 year mortality found there was no significant trend between baseline serum uric acid and CHD or CVD mortality.

A small cross sectional study analysed admission SUA levels in 240 patients undergoing coronary angiography. [35] There was no correlation between SUA and the extent of coronary artery disease ($P>0.05$). In

addition there was no significant difference between patients divided into CAD negative and CAD positive groups, with regards to uric acid level. Stepwise multivariant analysis using CAD as the dependent variable and factors of age, gender, BMI, smoking, blood pressure, LDL cholesterol, HDL cholesterol, triglycerides, total cholesterol and SUA levels as independent variables did not show any significant difference. (P>0.05)

A summary of the studies reviewed is noted in Table 2.

Study	Conclusion
NHANES I Follow up Study 1971-1992[24]	SUA levels independently and significantly associated with risk of cardiovascular mortality.
JCAD[25]	Elevated SUA is an independent predictor of cardiovascular events and all-cause mortality combined in patients with CAD.
LIFE[29]	Baseline SUA remained significantly associated with increased risk of cardiovascular morbidity and mortality in women only.
Zoppini et al[26]	Higher SUA associated with increased risk of CVD mortality in type 2 diabetics, independent of several potential cofounders.

Niskanen et al[30]	SUA strong predictor of CVD mortality in healthy middle aged men, independent of other variables.
Rotterdam[37]	SUA is a strong risk factor for MI and stroke.
Framingham[31]	SUA does not have a causal role in the development of CAD, death from CVD or death from all causes.
ARIC[32]	Findings not consistent with SUA being independent risk factor for CVD.
Jee et al[33]	Uric acid is not an independent factor for death from cancer, atherosclerotic CVD or all causes.
Persky et al[36]	For men the associations between SUA and mortality appear to be secondary to associations with uric acid and other risk factors.
Hashemi et al[35]	Uric acid is not significantly associated with coronary atherosclerosis.

Table 2 Summary of studies

There is therefore some evidence that uric acid may be an independent risk factor for cardiovascular disease and certainly convincing evidence increasing uric acid levels are associated with increased prevalence of

other cardiovascular risk factors. At present however the evidence remains conflicting and it is clear a large randomised controlled trial would be required to help answer the question.

1.3.3 Cellular Actions of Uric Acid

Hyperuricaemia has been linked to multiple pro-atherogenic processes including increased oxidative stress,[18, 38] vascular smooth muscle proliferation and plaque instability.[4, 27] Elevated uric acid is also found to induce a pro-inflammatory state and this correlates with increase in inflammatory markers such as CRP.[19, 21]

The secretion of inflammatory mediators and the inhibition of endothelial nitric oxide (NO) have been found to cause endothelial dysfunction. [29, 39] NO, synthesised by endothelial cells, prevents leukocyte and platelet aggregation, smooth muscle, lipid oxidation and elicits potent vasodilatory properties activating smooth muscle cells guanylate cyclase.[40]

NO inhibition may also contribute to the evolution of atherosclerosis[39] and it has been reported that atherosclerotic plaque contains a considerable amount of uric acid.[25]

Furthermore it has been suggested that elevated SUA levels are associated with increased platelet adhesiveness and this effect could potentiate thrombus formation in patients with acute coronary syndromes. [25, 29, 38]

Another role of uric acid is as a mediator for the development of other risk factors, particularly hypertension although the mechanism for this remains unclear. One potential mechanism is the stimulation of the renin angiotensin system.[38]

1.4 Oxidative stress

As described in section 1.1.3, oxidant by-products, such as superoxide anion ($O2^-$) and hydrogen peroxide (H_2O_2) are produced in the body as a consequence of normal aerobic metabolism. These ROS molecules are highly reactive with other biological molecules due to the presence of an unpaired electron.[41] Under normal physiological conditions the production of oxygen free radicals and peroxides is balanced by an efficient system of antioxidants which are molecules capable of scavenging ROS, thereby preventing oxidative damage.

In pathological states free radicals may be present in relative excess. These states may be due to endogenous sources or external stimuli such as toxins, ionising radiation and ultraviolet light. The shift of balance in favour of oxidation, termed oxidative stress may have detrimental effects on cellular and tissue function. Enhanced production of ROS is a major cause of endothelial dysfunction.[18] Free oxygen radical formation and release are also exacerbated by the activated neutrophils that congregate within ischaemic areas.[19] Furthermore oxidative stress and inflammation are closely linked.[42]

However the role of oxidative stress has been a much debated issue because attempts to reduce oxidative stress using antioxidant vitamins, such as in the Heart Failure Outcomes Prevention Evaluation (HOPE) study, have consistently failed to demonstrate a benefit.[18] Therefore more recent research has concentrated on mechanisms to reduce the formation of ROS rather than a scavenging approach to already formed ROS.

The two major ROS generating are the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and the xanthine oxidase systems. NAPDH oxidase catalyses the reduction of oxygen, through electron donation from either NADH or NADPH, to generate superoxide ($O2^-$). This system is thought to be the predominant driver of $O2^-$ formation. The most potent inducer of NADPH system is angiotensin II,[18] this effect can be reduced by the ACE inhibitors, angiotensin II receptor antagonists and direct renin inhibitors which are in frequent clinical use. Uric acid activates NADPH oxidase and inhibits nitric oxide.[19]

1.5 Allopurinol

1.5.1 Allopurinol and angina

The Clinical Outcomes Utilizing Revascularisation and Aggressive Drug Utilisation (COURAGE) trial research group found patients with stable CAD still had prominent rates of residual symptoms and impaired quality of life even in patients who had received optimum treatment. It is worth noting overall survival in angina is not usually improved by treatments which improve symptoms, but rather by those which reduce plaque formation and instability. With the exception of beta blockers, angina treatments which reduce symptoms are completely different from those which prolong survival. The former are nitrates, Ca antagonists etc while the latter are statins, aspirin and ACE inhibitors. Improving survival is thus achieved through various mechanisms including reduction of endothelial dysfunction – this is currently achieved with statins and ACE inhibition.[43]

Allopurinol has been shown in previous work to prolong the time to chest pain and ST segment depression during exercise in chronic stable angina.[44] 'It is known that myocardial ischaemia alters the redox state of the mitochondrial respiratory chain and ultimately promotes cellular adenosine triphosphate degradation, which leads to xanthine

accumulation and the subsequent increase in the substrate for xanthine oxidase'.[19]

One study found that patients on optimal standard therapy still exhibited marked oxidative stress and in this study XOR inhibition with allopurinol profoundly improved vascular tissue oxidative stress and vascular dysfunction in patients with coronary artery disease.[43]

It has been shown that allopurinol not only reduces urate levels but it also reduces oxidative stress and a number of mechanisms have been proposed as to why it would be useful in patients with CAD.

1.5.2 History and pharmacokinetics

Allopurinol was developed as an inhibitor of the enzyme xanthine oxidase. Although it is widely used to treat the hyperuricaemia associated with gout, it was initially used to inhibit XO catabolised metabolism of mercaptopurine,[17] thereby potentiating the action of this cytotoxic drug.

Allopurinol is an analogue of hypoxanthine (see figure 4). It is a weak acid with an acid dissociation constant (pKa) of 9.4 and is therefore essentially un-ionised at all physiological PH values.[17] The active metabolite is oxypurinol (see figure 5).

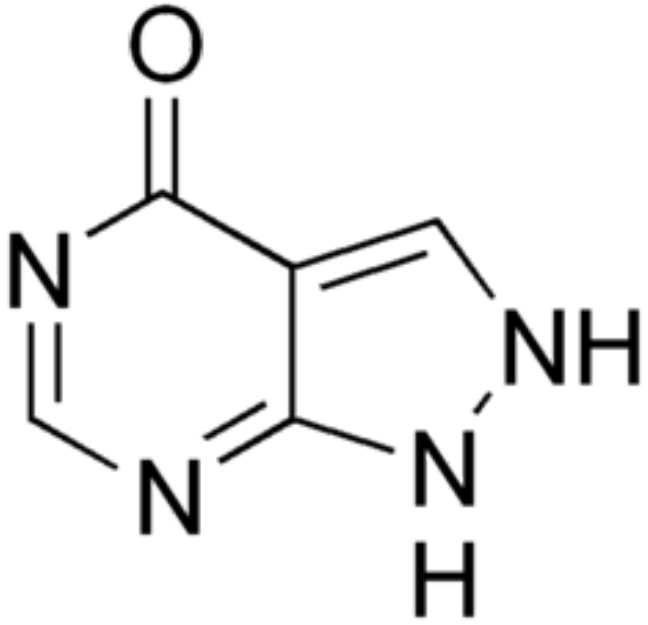


Figure 4 Structure of Allopurinol

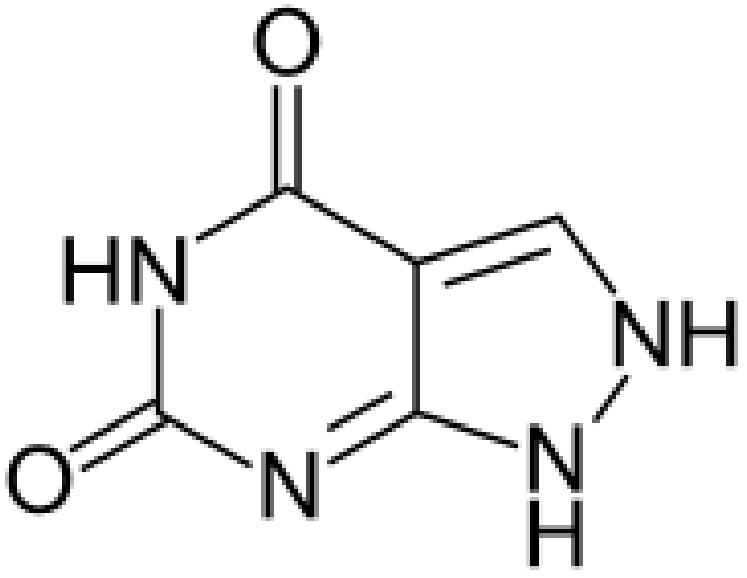


Figure 5 Structure of Oxypurinol

Allopurinol is largely metabolised to oxypurinol in the liver and excreted via the kidneys. After intravenous administration 12% +/- 6% (mean +/- SD) of the dose of allopurinol is excreted unchanged in the urine with 76% +/- 8% excreted as oxypurinol.[17] The half-life is 18-30 hours in patients with normal renal function.[45]

The standard dose initially is 100mg daily which can be titrated up to a maximum dose of 400mg daily. In patients with hepatic impairment the dose should be reduced and in patients with renal impairment the maximum dose is 100mg daily.[46]

Side effects include rash, gastro-intestinal disorders and rarely malaise, headache, vertigo, drowsiness, visual and taste disturbances, hypertension, alopecia, hepatotoxicity, paraesthesia and neuropathy, gynaecomastia and blood disorders. Allopurinol is also rarely associated with the development of Stevens-Johnson syndrome and toxic epidermal necrolysis.[47]

The basic mechanism of action of allopurinol and oxypurinol is inhibition of XOR. Allopurinol is a competitive inhibitor of XO at low doses and a non-competitive inhibitor at high doses. Oxypurinol is also a non-competitive inhibitor of XO.[19] This leads to decreased plasma concentrations of urate and elevated levels of the precursors,

hypoxanthine and xanthine. Hypoxanthine is converted to inosine, inosine monophosphate and closely related purine ribotides, adenosine and guanosine monophosphates.

1.5.3 Reduction of oxidative stress

Reducing oxidative stress has been shown to decrease myocardial oxygen demand per unit of cardiac output in patients with heart failure.[48] In experimental medicine it has been shown this is primarily due to the effect of xanthine oxidase inhibition on oxidative stress. In addition, two studies using the intra-arterial Vitamin C technique to assess vascular tissue OS, have shown clearly that allopurinol profoundly suppresses vascular tissue OS even in the presence of conventional optimal therapy. [49, 50] If allopurinol really does reduce XOR induced oxidative stress in human angina this effect may contribute to its anti-anginal pain effect. [43] A major caveat is that in large multicentre trials, antioxidant vitamins did not reduce CV events but this may well be because the doses needed to mop up OS effectively are much higher than can be given orally. In any case, blocking the formation of OS may be a better strategy as it is thought that at least some of the benefit seen by the use of both statins and ACE inhibitors is due to their ability to prevent OS forming.

1.5.4 Mechanoenergetic uncoupling

This is the term used to describe a decrease in the efficiency of contraction despite a constant oxygen cost of contraction. The mechanisms responsible for mechoenergic uncoupling are unknown although experimental medicine suggests ROS play a major role.[51] In animal models of heart failure it has been shown that XO inhibition improves myocardial efficiency and enhances the myocardial response to dobutamine and exercise.[51] A study by Ekelund et al[52] in which intravenous allopurinol 200mg was given to dogs with pacing induced heart failure in addition to a control group, found in both groups allopurinol resulted in increased myocardial contractility while reducing cardiac energy requirements. These findings were enhanced in the heart failure group.

In a small study[53] of patients with dilated cardiomyopathy (n=9) myocardial oxygen consumption, peak rate of rise of left ventricular pressure, stroke work and efficiency were measured at baseline and after sequential infusions of intracoronary allopurinol. Allopurinol was found to significantly decrease myocardial oxygen consumption without a parallel decrease in efficiency, stroke work or change in ventricular load. These effects were apparent despite concomitant treatment with

standard heart failure therapy including ACE inhibitors and beta-blockers.

1.5.5 Improvement of endothelial function

Enhanced production of ROS is a major cause of endothelial dysfunction[18] and allopurinol has the potential to improve endothelial function due to a reduction of oxidative stress which would in turn cause a reduction in vascular nitric oxide inactivation. In all patient groups studied, allopurinol was found consistently to improve endothelial dysfunction.[39, 43] This was shown to be due to reduced OS and not due to changes in uric acid.[39] This effect was also seen on top of conventional optimal therapy with statins and ACE inhibitors etc. This ability of allopurinol to improve endothelial dysfunction raises the possibility that it could be useful to prevent or slow the progression of atherosclerosis. Numerous clinical studies have shown XO inhibition improves endothelial function in patients with diabetes, CAD, smokers and chronic heart failure.[18 39, 43]

1.5.6 Improvement in coronary blood flow

Coronary endothelial dysfunction is associated with vasoconstriction rather than vasodilatation when acetylcholine (traditionally a vasodilator) is given. This effect extends to the coronary

microcirculation, the vasoconstriction contributes to myocardial ischaemia and subsequent angina.[19] A number of clinical studies have found inhibition of XO by allopurinol or oxypurinol improves endothelial dysfunction in the forearm in smokers, heart failure, diabetes mellitus and hypercholesterolemia. [54-56]

An open-labelled prospective non-randomised trial of patients with stable CAD, preserved left ventricular function and normal SUA levels, whom had no current allopurinol intake assessed the subject's coronary vasoconstrictor response to acetylcholine and changes in coronary blood flow after intravenous administration of oxypurinol. In patients with preserved coronary endothelial function, oxypurinol had no effect on acetylcholine-dependent changes in the arterial minimal lumen diameter. However in patients who displayed endothelial dysfunction as measured by a coronary vasoconstrictive response to acetylcholine, the study found significant improvement in coronary blood flow after administration of oxypurinol. This study concluded that XO derived free radicals greatly debilitated the availability of coronary nitric oxide in CAD and that XO inhibition as a treatment should be further investigated. [40]

1.5.7 Atherosclerosis and plaque stability

As described in section 1.1.5, atherosclerosis is a chronic disease characterised by 2 fundamental hallmarks namely lipid accumulation and inflammation. The interaction between these 2 processes defines the principal pathogenesis and distinguishes atherosclerosis from other chronic inflammatory disorders. Atherosclerosis is a progressive process in which lipids, extracellular matrix and activated vascular smooth muscle cells (VSMCs) accumulate in the arterial wall resulting in growth of an atherosclerotic plaque. The atheroma typically evolves into a more fibrotic and complex lesion. [57] It is known that inflammation plays a key role in atherogenesis and furthermore inflammation can elicit ACS.[58] Elevated uric acid can induce a pro-inflammatory state and this correlates with increase in inflammatory markers such as CRP.[19]

Many factors have been described which contribute to plaque instability in atherosclerotic disease. One factor is dysregulated metabolism of the extracellular matrix, principally due to focal overexpression of matrix metalloproteinases (MMP's) which may contribute to weakening of the atherosclerotic plaques. It appears the integrity of the fibrous cap depends on a dynamic balance between collagen synthesis and degradation.[57, 59] Inflammatory cytokines

regulate the expression of genes that direct interstitial collagen synthesis and MMPs which initiate breakdown of collage. Activation of MMPs seems to involve both inflammatory cytokines and oxidative stress. [57, 59]

Disruption of this balance results in plaque rupture which underlies as many as ninety percent of acute myocardial infarctions. The areas of the plaques prone to rupture are the 'shoulder areas' which often contain macrophages.[4] Colocalisation of macrophage foam cells and active forms of MMPs within these vulnerable regions is likely relevant for disruption of atherosclerotic plaques. There is a strong connection between plaque vulnerability and the presence of macrophages[4] however the mechanism whereby macrophages influence MMP activity remains poorly defined. One study found that macrophage foam cells, isolated from atheroma, steadily produced O_2-, H_2O_2 and NO without additional exogenous stimulation. The reactive oxygens were shown to module some MMP activity[4] and this may play an important part in modulation of MMP activity in atherosclerotic plaques.

MMPs also promote platelet activation. (See figure 6)

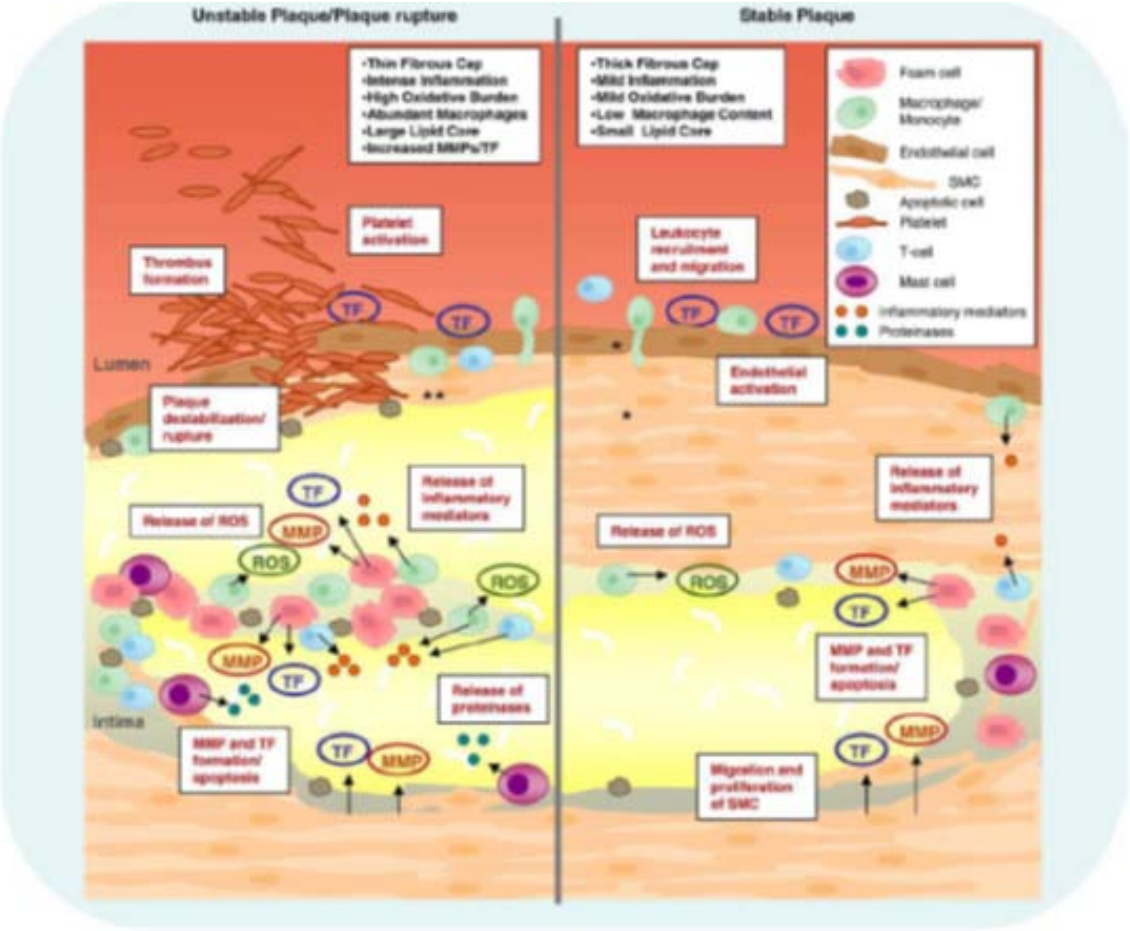


Figure 6 Features of stable and unstable plaques[57]

Allopurinol may have a beneficial role in establishing plaque stability by reducing the production of the reactive oxygen species which module MMP activity. Furthermore the ability of allopurinol to improve endothelial/vascular function and to profoundly reduce OS suggests a possible role for allopurinol to prevent or regress atherosclerosis, even in the presence of conventional optimal therapy (statins, ACE inhibitors).

1.5.8 Adenosine Triphosphate

Adenosine Triphosphate (ATP) is required for normal cardiac contractile function and has several roles:

- Fuel the ion pumps that directly remove calcium from the cytosol during diastole namely the sarcoplasmic reticular calcium-ATPase pump and the sarcolemmal calcium extrusion pump
- Provide energy for the sarcolemmal sodium pump (Na-K-ATPase). An active sodium pump is critical for achieving myocyte relaxation because the intracellular sodium concentration regulates intracellular calcium levels via sarcolemmal sodium-calcium exchange.
- ATP influences myocardial relaxation directly at the level of the myofilaments by interacting with actomyosin to cause its dissociation to actin and myosin, a step critical to relaxation[60]

XO plays a critical role in the terminal degradation of ATP. By blocking XO, allopurinol has the ability to prevent adenosine triphosphate degradation in ischaemic tissue.[19] There is also the potential that clinical benefit might also ensue from increasing the concentration of the substrates of XO namely hypoxanthine and oxygen, both of which should be increased by allopurinol blocking XO. ATP is broken down to

hypoxanthine and in theory; increasing hypoxanthine might increase ATP and thus energy. Indeed data do show that allopurinol does indeed increase ATP in the hypoxic rat heart (by 32%).[61] A study by Hirsh et al[62] randomised patients with non-ischaemic cardiomyopathy to receive allopurinol (300mg intravenously) or placebo infusion and the myocardial concentrations of ATP and creatinine phosphate and the rate of ATP synthesis through creatinine kinase were determined by magnetic resonance spectrometry. The study found allopurinol acutely improved the relative and absolute concentrations of myocardial high energy phosphates and ATP flux (see figure 6).

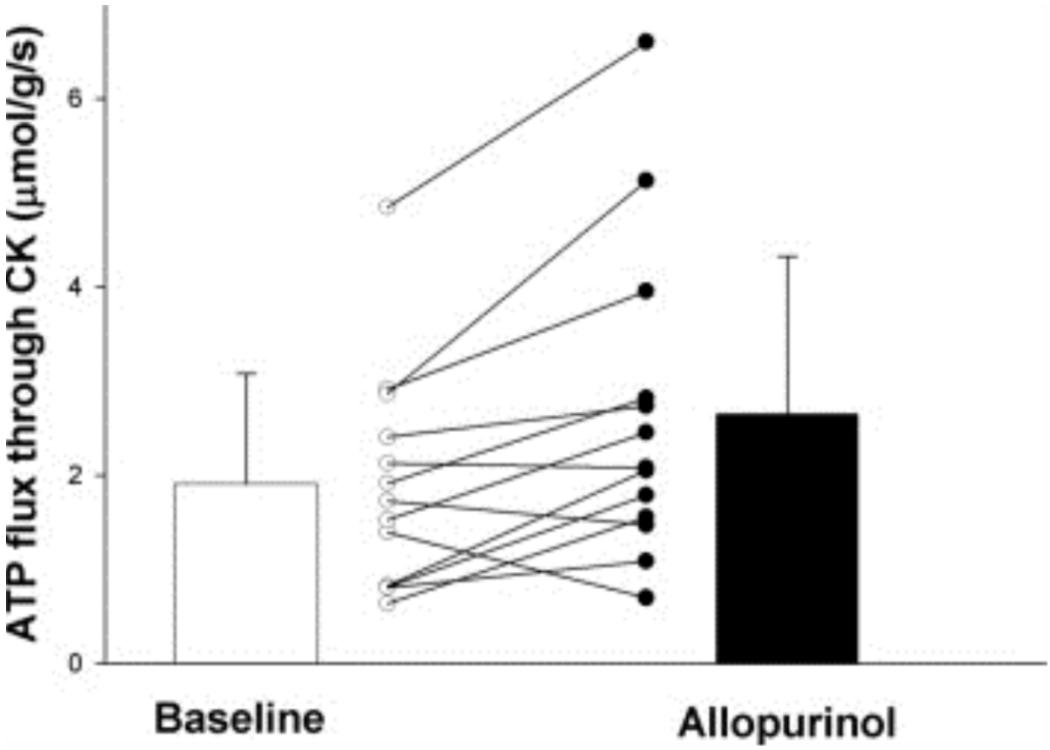


Figure 7 The rate of ATP synthesis through cardiac CK (µmol/g/s) under baseline and allopurinol conditions (summary bars represent mean ± SD).59T

The mechanism may in fact be more complex than described above and partly due to the effect of allopurinol on OS because OS inhibits cytosolic creatinine kinase which normally re-synthesises ATP.

Another substrate of XO which could be relevant is oxygen and it has been shown that allopurinol can improve myocardial oxygen consumption in heart failure.[51, 52] The mechanism of such an effect remains unknown but an increase in oxygen and/or ATP or both could be contributing.

1.6 Allopurinol in ACS

There is the suggestion that allopurinol may also have a role in acute coronary syndrome and this can be attributed to a number of mechanisms. OS increases after myocardial infarction and is thought to induce ventricular dilatation and dysfunction and impair endothelial function. [63] Therefore improvement in endothelial function by decreasing OS might improve coronary perfusion to downstream ischemic areas as well as reduce future atherosclerosis. Secondly the ability of allopurinol to reduce OS also raises the possibility that it might prevent plaque rupture which would be relevant to all acute ischemic CV events. Thirdly, more ATP and oxygen might prevent downstream ischemic cardiomyocytes from dying. To date one small study confirms that allopurinol can indeed reduce both troponin release and cardiac events after a ST elevation myocardial infarction.[64]

Another study found allopurinol treatment initiated immediately after coronary artery ligation in mice reduced cardiac remodelling, with a marked reduction in myocardial ROS production, suggesting XO inhibition might be beneficial in myocardial infarction[65].

1.7 Isoprostanes

While oxidative stress is now recognised to be a prominent feature of many diseases, definite evidence of this has often been lacking due to a shortcoming of methods available to assess oxidative stress in vivo in humans. Several in vitro markers of oxidative stress are available but most are of limited value in vivo because they lack sensitivity or specificity or require invasive methods. [66]

It has been established measurement of F2-isoprostanes is a reliable method to assess oxidative stress status in vivo.[66] These bioactive prostaglandin-like compounds are formed by free radical-catalyzed peroxidation of arachidonic acid and act as vasoconstrictors. Some studies have shown cyclooxygenase inhibitors increase the amount of isoprostanes and exacerbate the loss of cardiac function due to ischaemic-reperfusion injury.[67] Data has shown that allopurinol has the ability to significantly reduce F2-isoprostane levels in patients with high baseline oxidative stress.[19] There are several advantages to using isoprostanes as a measure of oxidative stress. They are chemically stable, formed in vivo, present in detectable amounts in normal tissue and biological fluids allowing definition of the normal range and are unaffected by the lipid content in the diet. One caveat is that F2-

isoprostane formation is impaired at elevated oxygen tensions such as hyperoxia induced lung injury.

1.8 Oxidised Low Density Lipoprotein (LDL)

Lipids, such as cholesterol and triglycerides are insoluble in plasma and are transported in lipoproteins to various tissues for energy utilisation, lipid deposition, steroid hormone production and bile acid formation. These lipoproteins are classified by their subclass size and concentrations. There are five major lipoproteins each of which has a different function.

- Chylomicrons – very large particles which carry dietary lipids.
- Very low density lipoproteins - carry endogenous triglycerides and cholesterol.
- Intermediate density lipoprotein - carry cholesterol and triglycerides.
- Low density lipoproteins – carry cholesterol
- High density lipoproteins – carry cholesterol

LDL particles contain a core of cholesterol esters, lesser amounts of triglycerides but are enriched by apolipoprotein B-100 which is the ligand for binding to the apo B/E receptor. LDL can be internalised by hepatic tissue for conversion to bile acids or by non-hepatic tissue for hormone production, cell membrane synthesis or stored in the

esterified form. The internalisation of LDL is regulated via negative feedback of the B/E receptor expression.

Chemically modified LDL such as oxidised LDL bypasses this negative feedback and is avidly taken up by macrophages and some other tissues through unregulated scavenger receptors. The oxidation process modifies a lysine amino acid on the apolipoprotein B and this process can occur within endothelial cells, macrophages, smooth muscle cells and T lymphocytes. The oxidation of LDL results in the formation of isoprostanes.

This pathway can result in excess accumulation of intracellular cholesterol and the formation of foam cells which contribute to the formation of atherosclerosis. Oxidised LDL can cause endothelial dysfunction, increased platelet aggregation and may have a role in plaque stability.[68-70]

A strong association between oxidised LDL and CAD has been shown by several studies[68, 71] with increasingly levels of oxidised LDL correlating with severity of CAD.[72] This was true after correcting for other CVD risk factors. Furthermore Meisinger et al[68] found in apparently healthy middle aged men, levels of oxidised LDL could predict those who would

subsequently develop an acute cardiac event even after adjustment for other major risk factors.

1.9 C - Reactive Protein (CRP)

CRP is an acute phase protein demonstrated to be a reliable measurement of inflammation. With emerging evidence on the role of inflammation in atherosclerosis, some studies have suggested a role for CRP in the oxidation of LDL and there is also evidence CRP is released from atherosclerotic lesions.[73] Furthermore CRP has a potential role as a marker of cardiovascular disease and has been found to give a predictive value beyond that of traditional risk factors in patients with CAD even after adjustment for conventional cardiovascular risk factors.[74-76]

1.10 B-type Natriuretic Peptide (BNP)

BNP is a 32-amino acid polypeptide which is released into the circulation from the myocardial membrane in response to myocardial stretching and increased left ventricular end diastolic pressure. BNP has an established role in the diagnosis and prognosis of left ventricular heart failure. Furthermore elevated BNP levels after acute myocardial infarction have a strong prognostic value.[77]

More recently it has been shown elevated levels of BNP at rest were independently associated with inducible ischaemia during ETT or stress echocardiography. The half-life of BNP is 20 minutes which means it may be possible to detect short term changes induced by exercise and indeed exercise induced ischaemia. A study by Win et al[77] found in subjects without heart failure, undergoing ETT, the BNP levels increased significantly post exercise and decreased towards baseline with 10-15minutes post exercise. This was true for patients with and without ischaemia but more pronounced in those with ischaemia.

A further study by Staub et al also found BNP levels increased significantly during acute myocardial ischaemia induced by dynamic exercise. This rise was not noted in angiographically normal subjects.[78]

A study by Paraskevaidis et al,[79] measured serial BNPs while exercising patients with preserved left ventricular function who were referred to outpatient clinic with chest pain. These patients then underwent coronary angiography. This study found BNP values were consistently increased in both the CAD and non-CAD groups (as determined angiographically) with the changes in the CAD group more pronounced. These findings were similar to those noted by Win et al. The study also found the degree of change was higher in patients with more severe CAD (see figure 7). Interestingly a further study by Davidson et al [80] found that although disease of left anterior descending artery were associated with increasing BNP levels as previously noted, the BNP levels were independently reduced by disease of the right coronary artery. They concluded this paradoxical effect of right coronary artery disease limited the value of BNP measurements as predictors of coronary disease severity.

Serial BNP disease measurements, before exercise, at peak, and 20 min after it, in patients with no CAD, with single, and multivessel disease.

Groups	BNP (pg/dl) before exercise	BNP (pg/dl) peak exercise	BNP (pg/dl) after exercise	*P* value
No CAD (*n*: 22)	14.2 ± 1 7.0	38.2 ± 51.1	51.8 ± 68.9	0.002
Single vessel (*n*: 41)	22.9 ± 15.5	59.0 ± 55.5	84.33 ± 90.4	<0.001
Multivessel (*n*: 37)	20.65 ± 15.22	82.05 ± 69.64	136.78 ± 137.2	<0.001
P value	0.02	0.001	0.001	

BNP, B-type natriuretic peptide; CAD, coronary artery disease.

Paraskevaidis I A et al. European Journal of Cardiovascular Prevention & Rehabilitation 2011;18:72-78

Table 3 Serial BNPs and severity of CAD

It has been suggested therefore that in patients with stable CAD, resting BNP values are associated with the presence of myocardial ischaemia and angiographic disease severity. Moreover it has been consistently shown BNP values are powerful predictors of death in patients with stable CAD or ACS.

1.11 Troponin T

The myocardial sarcomere consists of 7 actin monomers, double stranded tropomyosin and a troponin complex. The troponin complex is composed of 3 subunits – troponin T which anchors the complex to the tropomyosin strand, troponin C which binds calcium ions released from the sarcoplasmic reticulum and troponin I which inhibits the enzyme hydrolyses of ATP. Troponin is released from the myocardium in response to ischaemia. Troponin levels have an established role in the assessment of patients presenting with acute coronary syndrome as it is a specific marker of myocyte necrosis.

Troponin release has also been noted after exercise and a meta-analysis of 26 studies looking at athletes found post exercise troponin concentrations were detectable in approximately half the participants.[81] More recent studies found in an non-athletic population troponin was released after prolonged walking and the magnitude of the release was related to exercise intensity and cardiovascular pathology.[81]

1.12 ETT

ETT is the most widely used test in the evaluation of patients presenting with suspected CAD. It is well validated for establishing diagnosis and prognosis of CAD, as well as assessing exercise capacity. The sensitivity and specificity of ETT have been derived from studies correlating the ECG response to exercise with coronary angiographic data. Patient gender, age, coronary risk factors and the characteristics of the chest pain are also important determinants of the pre-test probability of coronary heart disease and therefore the diagnostic accuracy of ETT. [82] Sensitivity in men is 0.68 while in women it is 0.61, specificity is 0.77 in men and 0.70 in women.[6] The Duke score was developed to predict prognosis. (see figure 8)

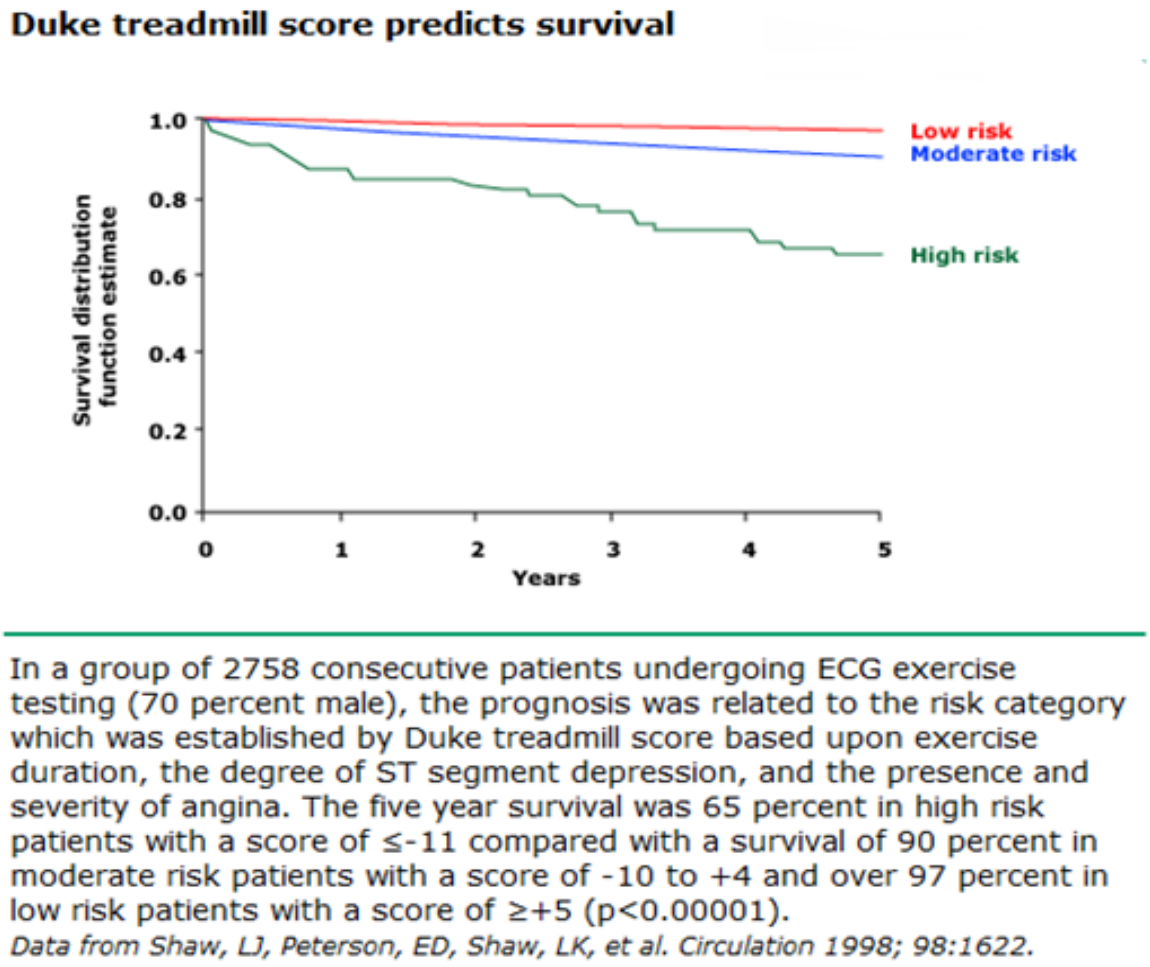


Figure 8 Duke Score and Outcome

ST depression during exercise is one the main ECG change used to assess ischaemia. The test is considered positive when there is ≥1mm horizontal or down-sloping ST depression in one or more leads that persists at 80milliseconds after the J point. (see figure 9 and 10)

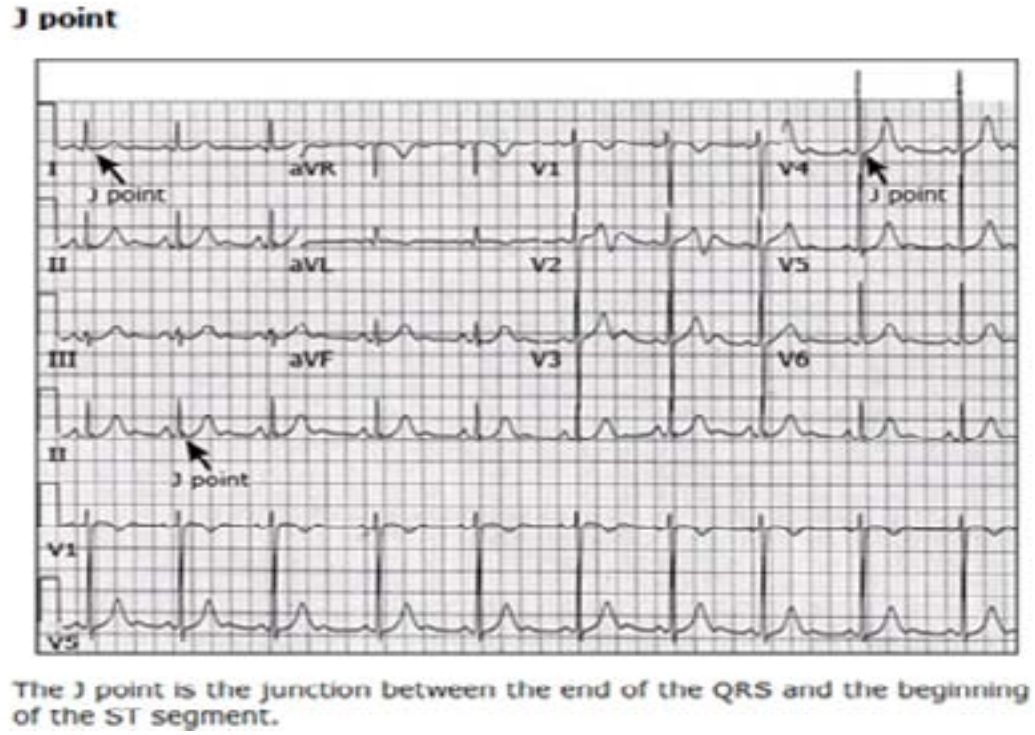


Figure 9 Example of J point

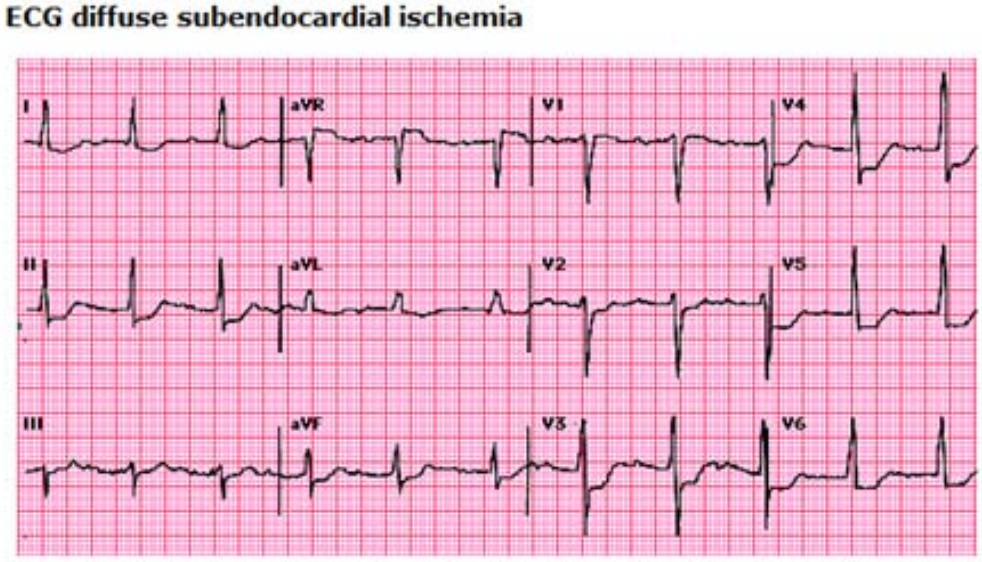


Figure 10 Example of ST depression

Indications for ETT

ETT can be used to assess patients with:

- Symptoms suggesting myocardial ischaemia
- Acute chest pain in who ACS and MI have been excluded
- Recent ACS treated without angiography
- Prior coronary revascularisation
- Valvular heart disease
- Newly diagnosed heart failure or cardiomyopathy
- Certain cardiac arrhythmias

Contraindications for ETT

- Acute myocardial infarction
- Unstable angina
- Uncontrolled arrhythmia causing symptoms of haemodynamic compromise
- Symptomatic severe valvular stenosis
- Uncontrolled symptomatic heart failure
- Active endocarditis
- Acute aortic dissection
- Acute pulmonary emboli

There are 2 main groups of patients in whom ETT is limited. Firstly, in those who due to a physical limitation, are unable to exercise sufficiently. Secondly patients with ECG changes at rest which can interfere with the diagnosis of ischaemia.

There are a number of reasons an ETT may be stopped. Firstly patient determined reason such as the patient wishes to stop, has significant chest pain, marked fatigue or shortness of breath or any other limiting factors. Secondly the supervising doctor may stop the test if the patient looks unwell, develops exertional hypotension, exertional hypertension (systolic BP >200, diastolic BP >100). Thirdly several ECG endpoints would require the test to be stopped including marked ST depression, new bundle branch block, new high grade heart block, ventricular tachycardia or fibrillation, increasing ventricular ectopy and onset of supraventricular tachyarrhythmia. Finally the test end may be protocol determined ie target heart rate achieved or workload determined.

1.13 Summary

This three arm cross over, double blind trial was designed with aim of assessing the role of allopurinol on patients with ischaemic heart disease, in particular to establish time of onset of effect of the drug and the optimal dose. George et al [39] demonstrated that a steep dose–response relationship existed between allopurinol and its effect on endothelial function. If the onset of allopurinol was found to be rapid it may be required to be started quickly in patient with ACS to obtain maximum benefit. Patients with chronic stable angina were used as surrogate of the acute coronary syndrome, since they are easier to study as a group, to answer key questions of the speed on onset of allopurinol and the optimal dose. This would then inform potential study of allopurinol in ACS patients.

2 Methods

2.1 Overview

This study was a randomised double-blind, placebo-controlled, three arm cross over trial, run in accordance with the Declaration of Helsinki and The Medicines for Human Use (Clincal Trials) (Amendment) Regulations 2006.

The aim of the study was to determine whether allopurinol prolonged the time to ST depression on exercise treadmill test in patients with chronic stable angina and if so to establish how quickly this effect was noted and the optimum dose to achieve this.

2.2 Approvals

Sponsor Approval

Reference 2010CV30

Submitted 30th August 2011

Sponsorship was confirmed and permission to apply for CTA granted on the 7th October 2011.

Ethical Approval

Reference 11/ES/0033

Submitted 11th October 2011

An favourable reply was received from East of Scotland Research Ethics Service REC 2 on 11th November 2011 provided further information was submitted. A letter providing this was submitted on 15th November 2011 and a final approval received 22nd November 2011.

NHS R&D Approval

Reference 2010CV30

Submitted 18th October 2011

Approval was granted 12 January 2012

MHRA Approval

Reference 21726/0277/001/0001

Submitted 24th November 2011

The application was accepted on 23rd December 2011.

Trial Registries

ClinicalTrials.gov: the study was registered and allocated reference NCT01457820

EudraCT: the study was registered and allocated reference 2011-004966-35

Funding

Reference PG/11/14/28774

An application for funding was submitted to the British Heart Foundation on 25th October 2010. This was considered at the Projects

Grants Committee and an award covering the full costs was awarded on 31st March 2011.

2.3 Study Protocol

Individuals aged 18 to 85 years were recruited from outpatients at two Tayside Hospitals (Ninewells Hospital and Perth Royal Infirmary) and via the SPCRN.

2.3.1 Inclusion Criteria

Participants were recruited if they fulfilled the following inclusion criteria

- Aged 18 – 85 years
- A positive ETT – this was defined as development of ST depression ≥ 1mm at 80ms after the J point compared to baseline in at least one lead.
- A history of symptoms of chronic, stable, effort-induced angina for ≥ 2 months – patients with unstable angina or rest pain were excluded as they would be high risk for the repeated ETTs required in this study.

All concomitant antianginal medication were allowed and continued unchanged during the study.

2.3.2 Exclusion Criteria

- the inability to do an ETT due to back or leg problems
- myocardial infarction or acute coronary syndrome ≤ 2 months – these patients may be unstable and will be undergoing optimisation of standard therapy during this time.
- coronary revascularization (percutaneous or CABG) ≤ 6 months – patients take up to six months to fully recover from bypass surgery and the risk of in-stent stenosis after percutaneous intervention is highest in the early months following the procedure.
- Left ventricular ejection fraction <45% - repeated ETT's in patients with heart failure carry a higher risk.
- estimated GFR <60 ml/min or creatinine >180 mmol/ml – as allopurinol is renally excreted impaired renal function may lead to an increased frequency of side effects.
- significant valvular pathology – patients with valve disease are at higher risk during ETT.
- already had gout or on allopurinol – this would make findings difficult to interpret.

- atrial arrhythmias or ECG abnormalities interfering with ST-segment interpretation – as these would make the ETT difficult to report.
- previous ventricular arrhythmias on ETT – as these would make the patient high risk for repeated ETT.
- severe hepatic disease – allopurinol is contraindicated in these patients.
- on azathioprine, 6 mercaptopurine or warfarin – as these drugs interact with allopurinol.
- Patients who have participated in any other clinical trial within the previous 30 days will be excluded
- Patients who are unable to give informed consent will also be excluded from this trial

2.4 Cohort size and power calculation

Power calculations were carried out by a statistician, Dr Simon Ogston, using the real data from our previous study of allopurinol in chronic stable angina. [83] In that study, where n=60, the standard error (SE) of the estimated effect on ST depression during exercise was 6.75s (derived from 58-31/4). Therefore, the standard deviation of differences was 52.3s (derived from SE x $\sqrt{60}$). This meant in a target number of 60 individuals, we would have 80% power to detect a different effect size between the different doses of 18.9 secs (derived from (1.96 + 0.84) x 52.3/$\sqrt{60}$). A difference of 18.9 seconds at peak drug effect between different doses of allopurinol would represent an 8% change over the patients' baseline time to ST depression (232 seconds) in our previous study. [83] A number of 60 subjects would make our study appropriately sensitive to detect clinically significant differences between the various doses of allopurinol. Allowing for a 10% drop out rate we intended to recruit 66 participants.

2.5 Recruitment

Recruitment proved to be highly challenging in this study. A number of methods were used to identify potential participants.

2.5.1 The Scottish Primary Care Research Network (SPCRN)

Participants were recruited using the East of Scotland node of the SPCRN. This network was established in 2002 to help coordinate national research activity in primary care. It is funded by the Chief Scientists Office, centrally managed by the Scottish School of Primary Care and operationally managed at a regional level in Scotland.

The co-ordinator for the East of Scotland contacted GP practices within the network by post to invite them to be involved in the study. Practices willing to be involved were visited by a SPCRN research officer who screened their patient database electronically using the patient eligibility criteria to generate a list of potential participants. This list was then screened by a GP in the practice to exclude patients who may have reason not to be invited.

A letter of invitation (see Appendix A) was then posted out to the final list of potential participants on the practice headed paper along with a copy of the patient information sheet (PIS) (see Appendix B), a reply slip (see Appendix C) and a stamped addressed envelope. Individuals interested were invited to reply by post or by telephone to FS. Patients were then contacted via telephone and an appointment booked for screening. A confirmation of appointment was sent by letter where necessary (see appendix D)

2.5.2 Cardiology Outpatients

At the beginning of the study, general cardiology outpatient clinics in Ninewells were screened to identify suitable patients but it became clear patients attending general outpatient cardiology clinics did not fit the criteria. Details of all patients attending the rapid access chest pain clinic who were found to have a positive exercise tolerance test were forwarded to FS by the secretary for this clinic.

2.5.3 Angiography Database

A list of patients attending for angiograms over a six month period was generated by the radiology department in Ninewells. This list was then

further screened using the clinical portal on the NHS system by a research nurse. Patients found to be for medical management or with residual disease were identified and letters of invitation were sent containing PIS and reply slip as above to these individuals.

2.5.4 Informed Consent

All patients had received the PIS at least 24 hours in advance of attending for their screening visit. The programme of the study was outlined and all had the opportunity to ask questions prior to signing a written consent form (see Appendix E).

2.6 Study Visits

All study visits were carried out at the Department of Clinical Pharmacology, Ninewells Hospital, Dundee or the outpatient cardiology department, Perth Royal Infirmary.

Following written informed consent, the screening visit was completed. This involved an initial clinical assessment comprising

- Check of inclusion/exclusion criteria
- Past medical history
- Family history
- Social history
- Examination
- Height
- Weight
- Resting pulse and blood pressure
- Record of concomitant medications

Patients also had baseline safety bloods drawn.

A baseline ETT was then performed. Participants had to demonstrate ischaemia defined as ST depression of ≥1mm ST depression compared to their baseline ECG. Participants who did not demonstrate ischaemia were excluded from the study.

Participants who demonstrated ischaemia underwent a second ETT within 2 weeks of the first ETT. Eligible participants had to demonstrate ischaemia on both visits with a variability in time to ST depression of <15%. Otherwise a third ETT was performed (within 14 days of the second ETT) and the above criterion of time to ST depression was applied between the second and the third ETT.

This ETT criterion was set to exclude patients with a wide variation in the time to ST depression on repeated ETTs. Similar criterion has been used in other trials assessing anti-anginal agents.[84-86]

Participants then proceeded to the first randomisation visit. Another baseline ETT was performed one hour before the participant was dosed with either allopurinol 800mg or allopurinol 400mg or matched placebo. An ETT was performed 4 hours after the loading dose administration. Participants were then given a sealed bottle and asked to take one tablet twice a day for 5 days thereafter. The medication for this 5 day period was either allopurinol 400mg BD if started on 800mg loading dose, or 300mg BD if started on the 400mg loading dose, or matched placebo BD. Compliance was checked and documented using tablet counts at each visit.

The patient then returned the following day for another ETT and again 4 days +/- 1day for a further ETT. A washout period of a minimum of seven days occurred before the patient entered the second then third arm of the trial. Each arm was identical in format apart from the dose of allopurinol/placebo.

A full visit schedule is provided in table 4.

- Visit 1 (week 0) – screening visit 1
  - Participant consent – answer any outstanding questions and complete consent form.
  - Baseline ETT
  - Safety bloods
  - Record list of current medications
  - Record of medical history & CAD history

- Visit 2 (week 1) – screening visit 2
  - Second baseline ETT – if stable (<15% variance) then can continue in study
  - Record list of current medications

- 1-4 week screening to randomisation period

- Visit 3 – Randomisation visit (Day 0)
  - ETT one hour before study drug dosing
  - Dosing with Allopurinol/Placebo

- 2nd ETT after 4 hours of study drug dosing
- Record list of current medications
- Supply of full dose study medication x 5 days
- Safety and research bloods.
- Issue angina diary to participants

- Visit 4 progress visit (Day 1)
  - ETT
  - Monitor AEs
  - Safety and research bloods
  - Review angina symptom diary

- Visit 5 – progress visit (Day 5 +/- 1 day)
  - Assess medication compliance.
  - ETT
  - Check for AEs
  - Safety and research bloods.
  - Review angina symptom diary

- 1-4 week washout period

- Visit 6 - Randomisation visit (Day 0)
  - ETT one hour before study drug dosing
  - Dosing with Allopurinol/Placebo
  - 2nd ETT within 4 hours of study drug dosing
  - Record list of current medications
  - Supply of full dose study medication x 5 days
  - Issue angina diary to participants
  - Safety and research bloods.
  - Check for AEs

- Visit 7 – progress visit (Day 1)
  - Assess medication compliance.
  - ETT
  - Check for AEs

- Safety and research bloods.
- Review angina symptom diary

- Visit 8 – progress visit (Day 5 +/- 1 day)
  - Assess medication compliance.
  - ETT
  - Check for AEs
  - Safety and research bloods.
  - Review angina symptom diary

- 1-4 week washout period

- Visit 9 Randomisation visit (Day 0)
  - ETT one hour before study drug dosing
  - Dosing with Allopurinol/Placebo
  - 2nd ETT within 4 hours of study drug dosing
  - Record list of current medications
  - Supply of full dose study medication x 5 days

- Issue angina diary to participants
- Safety and research bloods.
- Check for AEs

- Visit 10 – progress visit (Day 1 day)
  - Assess medication compliance.
  - ETT
  - Check for AEs
  - Safety and research bloods.
  - Review angina symptom diary

- Visit 11 – final visit (Day 5 +/-1 day)
  - ETT
  - Assess medication compliance.
  - Safety and research bloods.
  - Check for AEs
  - Record list of current medications

Table 4 Visit schedule

2.7 Randomisation

Double blind medication (allopurinol or placebo) was prepared and packaged by Tayside Pharmaceuticals. The medication was labelled in sequential numbers and distributed to the participant by the research fellow according to their sequence number. Randomisation was carried out by Tayside Pharmaceuticals using block randomisation in eleven groups of six (with three active/three placebo in each block). A validated randomisation program was used and securely backed up containing both the randomisation seed and the randomisation allocation. The randomisation key was held in sealed enveloped by Tayside Pharmaceuticals, Ninewells, who operate a 24 hour emergency unblinding facility. An additional copy was stored in a locked fireproof box accessible by authorised University of Dundee staff not connected with the study.

At the time of patient proceeding to randomisation a letter was sent to the participant's GP (See appendix F) and a copy filed in their medical notes along with a copy of their signed consent form and contact details for the PI.

2.8 Outcome measurements

2.8.1 Primary and secondary outcomes

The primary outcome was to determine if allopurinol, of different doses, induced a change in time to exercise induced ST depression in patients with CSA when compared to placebo and, if so, when that effect comes on.

Secondary outcomes were:

- To determine if there was a change in total exercise time on ETT, at different doses of allopurinol in CSA patients compared with placebo.
- To determine if there was a difference in time to CSA patients reported symptoms of chest pain during exercise testing with different doses of allopurinol compared with placebo.
- To determine if there were changes in blood markers, specifically BNP, high sensitivity Troponin T and hsCRP, during exercise testing in patients with CSA at different doses of allopurinol compared with placebo.
- To assess, using self-reporting diaries, if there were differences in CSA patient's symptoms of angina pain and GTN usage when

treated with different doses of allopurinol compared with placebo.

2.8.2 Exercise tolerance test

These were conducted using the Bruce protocol (see table 2) which is the most widely used protocol in the assessment of patient with suspected or documented coronary artery disease. The protocol is divided into successive three minute stages each of which requires the patient to walk faster and at a steeper gradient.

Stage	Grade (%)	Speed (mph)	Total Time (min)	METS
1	10	1.7	3	4.5
2	12	2.5	6	7
3	14	3.4	9	10
4	16	4.2	12	13

Table 5 Bruce Protocol for ETT

The machine used was a GE Marquette Series 2000 Treadmill Stress Testing System (GE Healthcare Clinical Systems (UK) Ltd, Hatfield, UK).

The tests were conducted in accordance with the University of Dundee SOP for ETTs and supervised by FS (PI, Cardiology Registrar and Advanced Life Support Instructor) and a trained nurse. Patients had blood pressure checked at baseline, every three minutes throughout the ETT and repeated during the recovery period to ensure it was settling back to baseline. ECG monitoring was continuous throughout the test and during the recovery phase until the ECG returned to baseline and blood pressure was satisfactory. Full resuscitation equipment was available at all times. 12-lead ECGs were printed at 30 second intervals and more frequently at the point of 1mm ST depression.

2.8.3 Angina Diary

Patients were issued with an angina diary (see Appendix F) and asked to complete this throughout the study period. They were reminded at all visits to complete the diary. The aim of the diary was to document if the frequency of the angina attacks varied on different arms of the trial. The diary included a section for the date, number of attacks on a given day, the trigger for the attack, the severity of attack which was rated 1-4 (1-mild, 2-somewhat strong, 3- severe, 4-very severe), the duration of the attack and the intervention required to resolve the angina.

2.8.4 Laboratory tests

Safety Bloods – Full blood count, urea & electrolytes and liver function tests

A total of ~ 9 ml of venous blood was drawn at the patients initial visit with 5 ml serum for biochemistry and 4ml EDTA for haematology. These tubes were labelled using the ICE requesting system and hand delivered to the NHS blood sciences lab on level 7, Ninewells where automated analysis was carried out to NHS Tayside standards.

Patients randomised into the trial had these safety bloods taken and analysed at every visit. Any abnormal result was acted upon within 24

hours of the sample being drawn and the results were documented in the CRF.

The hard paper copy was annotated with the patient ID number and the name and hospital identification number were blacked out. All paper copies were stored with the CRF.

Test	Normal reference range
Haemoglobin	13.0-18.0 g/dL (men), 12.0-16.0 g/dL (women)
White cell count	4.0-11.0 $x10^9$/L
Platelets	150-400 x 10^9/L
Sodium	133-146 mmol/L
Potassium	3.5-5.3 mmol/L
Urea	2.5-7.8mmol/L
Creatinine	62-106 µmol/L
Alanine Transaminase (ALT)	5-35 U/L
Alkaline Phosphatase (Alk Phos)	30-130U/L
Bilirubin	0-21 µmol/L
Albumin	35-50 g/L

Table 6 Reference Ranges for Safety Bloods

Study Bloods - Uric Acid, Troponin T, CRP, Oxidised LDL and Isoprostanes.

Patients randomised had study bloods taken at every visit.

At all visits, baseline bloods were drawn of ~ 25ml of venous blood which was split into 2 x 10ml ETDA tubes and 5ml serum tube. (These samples were drawn at the same time as the safety bloods). The study tubes were stored on ice then centrifuged for 10 minutes at 3000rpm at 4ºC. The EDTA plasma was extracted and put into microtubes individually labelled for isoprostanes and oxidised LDL. These samples were the stored at -70 ºC. The serum was extracted and put into microtubes individually labelled for CRP, Troponin T and uric acid. These were stored at -20 ºC.

At visits 3, 6 & 9 a further ~5ml serum sample was drawn 2 hours after the loading of the study drug. This sample was handled in the same way as the serum sample above and stored for uric acid.

A ~25ml sample was then drawn before and after the second ETT of visits 3, 6 & 9 and these samples were handed as the baseline bloods.

For visits 4, 5, 7, 8, 10 & 11 the baseline sample was taken as described above and a second ~25ml sample of venous blood was drawn

immediately after the ETT on these visits. These bloods were also handled in the same way as the baseline samples described about.

All bloods were analysed blindly by the Immunoassay Core Lab, Cardiovascular and Diabetes medicine, University of Dundee, Ninewells Hospital.

Study Bloods - BNP

BNP was analysed by FS or the study nurse using the bedside Biosite Triage Meter. Alere BNP testing kits were stored in the cold room and removed 15 minutes prior to use as per the SOP. A 1ml of blood was removed from the EDTA tubes used for oxidised LDL and isoprostanes prior to these being centrifuged. This blood was pipetted onto the testing kit and the analysis ran. The machine gave a print out of the result which was dated and recorded in the paper CRF. If a sample was not available this was recorded and a sample of plasma from the corresponding visit was analysed by the Immunoassay Core Lab. BNP was analysed at all points oxidised LDL and isoprostanes was as recorded above.

2.9 Data entry and managment

Data from all study visits were recorded in the paper clinical record form (CRF) (see Appendix G) by either FS or the nurses working in the trial and recorded on the delegation log. All ETT print outs were signed, dated and numbers and stored along with the CRF in locked cabinets in the PI's office. The anonymised bloods results and completed angina logs were stored along with the ETTs and CRFs.

Electronic storage of the data was on the Openclinica system. There were significant delays in this being available and therefore the majority of the data were entered retrospectively. Data was double-data entered by FS and the study nurse. Data entry was double checked by an independent person with no links to the trials who ensured all data entered was consistent with data in CRFs.

2.10 Statistical analysis

The statistical analysis plan was drawn up by Daniel Levin, university of Dundee statistician. The main analysis was on an intention-to-treat basis.

2.11 Adverse Events

Participants were asked about adverse events at every visit and any adverse event was documented in the paper CRF. Any serious adverse event (SAE) or serious adverse reaction (SER) was reported to the sponsor in accordance with The Medicines for Human Use (clinical trials) Amendment Regulations 2006.

3 Results

3.1 Recruitment

As noted in section 2.5.1, the majority of patients were identified via the SPCRN network with a small number from out-patient clinic and the angiography database. Patients identified by the SPCRN network were further screened via GP databases by SPCRN staff to ensure eligibility before invitation letters were sent out. Patients identified from cardiology outpatients and the angiography data base were further screened via NHS Tayside clinical portal before invitations were sent out.

22 practices were screened by SPCRN with a total of 2492 invites send to patients. 122 patients accepted via this process, with 38 patients declining the invitation.

Patients replied by posting back reply slips in prepaid envelopes or by telephoning the department. They were called by the PI and further screened over the telephone before being invited for face to face screening.

A total of 121 patients were screened with 26 (21%) proceeding to randomisation. The high failure rate in passing screening was largely

due to insufficient ECG changes on the ETT with 57 (60%) patients not having sufficient changes to meet study criteria. A further 3 patients (3%) found it too difficult to walk on the treadmill, 3 (3%) developed severe hypertension on the ETT, 1 patient was excluded as their renal function was below the threshold for inclusion, 1 patient withdrew from screening as they had a chest infection and a final patient was excluded as there was a finding of a new murmur on the initial clinical examination.

The recruitment period was extended, as recruitment was challenging, for a further six month period. In addition we gained approval to extend the trial into NHS Fife, increasing the pool of patients.

The first screening visit occurred 9th May 2012 with the final visit 7th November 2014.

Five patients did not complete the study post randomisation. One patient injured his knee and was unable to continue on the treadmill, one patient felt the intensity of the study was too much for him and withdrew after the second arm, another withdrew as she did not like the frequency of the blood tests, a fourth patient withdrew on the last visit as he has developed a rash and a final patient was removed from the trial following a SUSAR.

Due to the complexity of the data collected in this study the statistical analysis was performed by statistican Daniel Levin.

3.2 Baseline Characteristics

Variable	Number	%
Gender (Male)	20	77
Cardiology exam abnormal	2	8
Respiratory exam abnormal	0	0
Abdominal exam abnormal	0	0
Neurology exam abnormal	0	0
Family history coronary disease (Yes)	15	58
Father (Yes)	8	31
Mother (Yes)	7	27
Brother (Yes)	6	23
Sister (Yes)	4	15
Previous smoker	14	54
Retired	22	85
Living Alone	3	11.5
Angina	26	100
MI	9	35
Stroke	1	4
Hypertension	9	35
Hypercholesteraemia	11	42

Table 7 Patient characteristics

Variable	Number missing	Mean	SD	Median	Min	Max
Age	0	70.1	6.2	69.5	61	83
Height	0	1.69	0.07	1.68	1.51	1.81
Weight	0	81.6	14.0	81.4	55.3	117.6
BMI	0	28.6	4.2	28.4	22.4	40.7
Pulse	0	70	12.9	69	51	93
Systolic	0	135.7	20.1	140	98	168
Diastolic	0	76.5	11.0	79	60	100
BNP (Predose, visit 3)	0	51	43	33	2.5	140
HB g/dL (visit 3)	2	14	1.4	13.85	11.7	16.5
WCC $x10^9$/L (visit 3)	2	6.7	1.8	6.35	4	10.3
PLTS $x10^9$/L (visit 3)	2	224	49.5	218	138	376
Na mmol/L (visit 3)	0	140	1.8	140.5	136	144
K mmol/L (visit 3)	0	4.3	0.32	4.3	3.5	5
Urea mmol/L (visit 3)	0	6.2	1.4	5.9	3.3	8.6
Creat µmol/L (visit 3)	0	77	12.5	75.5	55	110
ALT 5 U/L (visit 3)	1	27	11.4	25	11	63
Bilirubin µmol/L (visit 3)	1	9	3.4	8	2.5	15
Alk Phosphatase U/L (visit 3)	1	77	18	77	38	114
Albumin g/L (visit 3)	1	38	2.6	39	32	41
Uric acid	0	424.6	143.7	403.1	217.6	898.7
Of 14 ex-smokers:						
Cigarettes Per day	0	33.8	17.3	35	10	60
Yrs smoked	0	23.2	10.8	25	5	48
Yrs stopped	1	18.8	12.6	24	2	45
Yrs since diagnosis:						
Angina (25 pts)	5	13.2	8.1	11.5	2	32
MI (9 pts)	2	16.3	12.2	12	5	40
Stroke (1 pt)	0	17	-	17	17	17
Hypertension (9 pts)	8	16	-	16	16	16
Hypercholesteraemia (11 pts)	11	-	-	-	-	-

Table 8 Patient characteristics

Table 9 shows the number of patients on cardiac medications.

Medication	Number of Patients	Percentage
Beta-blocker	21	81
Ace-inhibitor	14	54
Angiotension inhibitior	3	12
Calcium channel blocker	9	35
Statin	23	88
Nitrate	14	54
Aspirin	23	88
Diabetic medication	4	15

Table 9 Patient Medications

Angiography results were available for 20 subjects. Table 9 shows the distribution of coronary artery disease in these subjects. LMS is the left main stem, LAD is left anterior descending artery, LCx is the left cicumflex and RCA is the right coronary artery.

	No disease	Plaque disease	25-50% stenosis	50-74% stenosis	74-94% stenosis	>95% stenosis
LMS	16	2	2	0	0	0
LAD	4	5	1	1	3	6
LCx	7	5	1	3	1	3
RCA	5	2	4	2	5	2

Table 10 Table of distribution of coronary artery disease

The number of patients with significant (>50% stenosis) single, double or triple vessel disease is detailed in table 10.

Vessels with >50% stenosis	0	1	2	3
Number	6	6	4	4

Table 11 Number of vessels involved

Five patients had undergone coronary artery bypass grafting and eight had a history of percutaneous coronary intervention.

3.3 Adherence to medication

Patients were asked to bring their medication with them to visits and these were counted to ensure compliance. The tablet count indicated patients were 100% compliant. Furthermore uric acid levels were checked at every visit and, as shown in figures 11-13, the uric acid dropped significantly in both treatment arms of the trial but not in the placebo arm which is consistent with compliance.

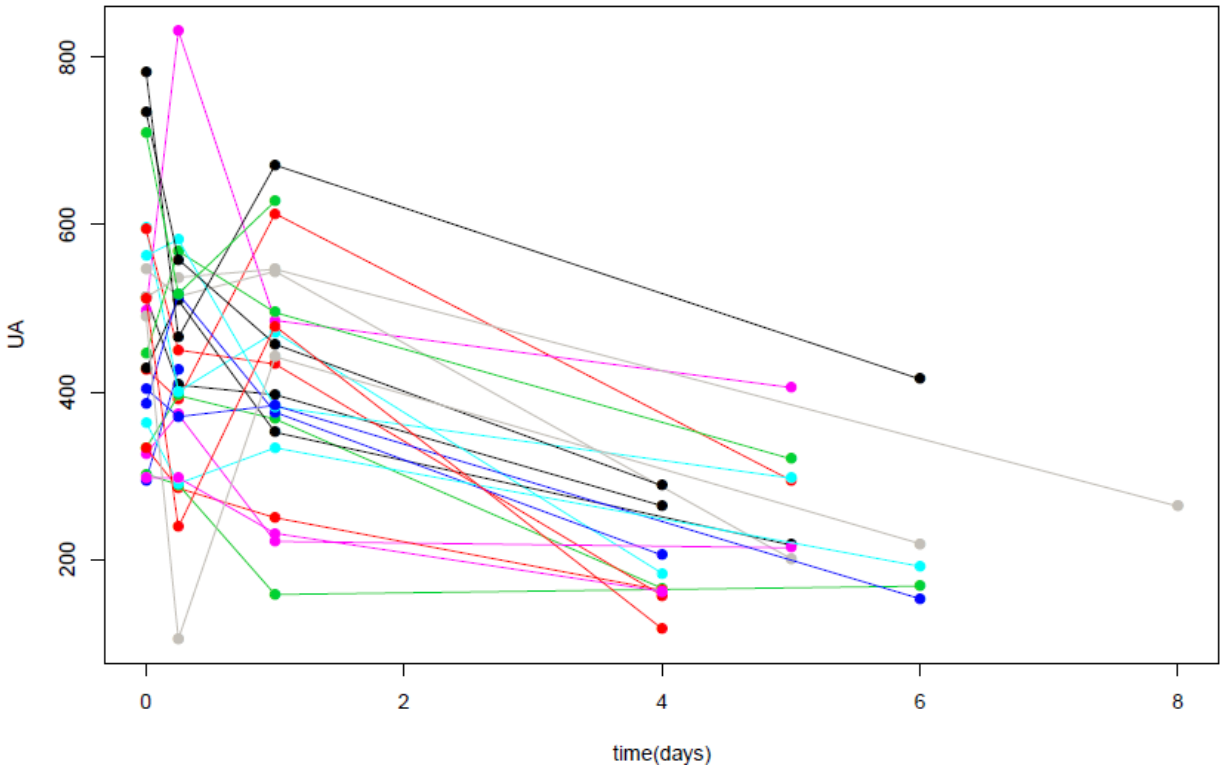


Figure 11 Uric acid levels low dose

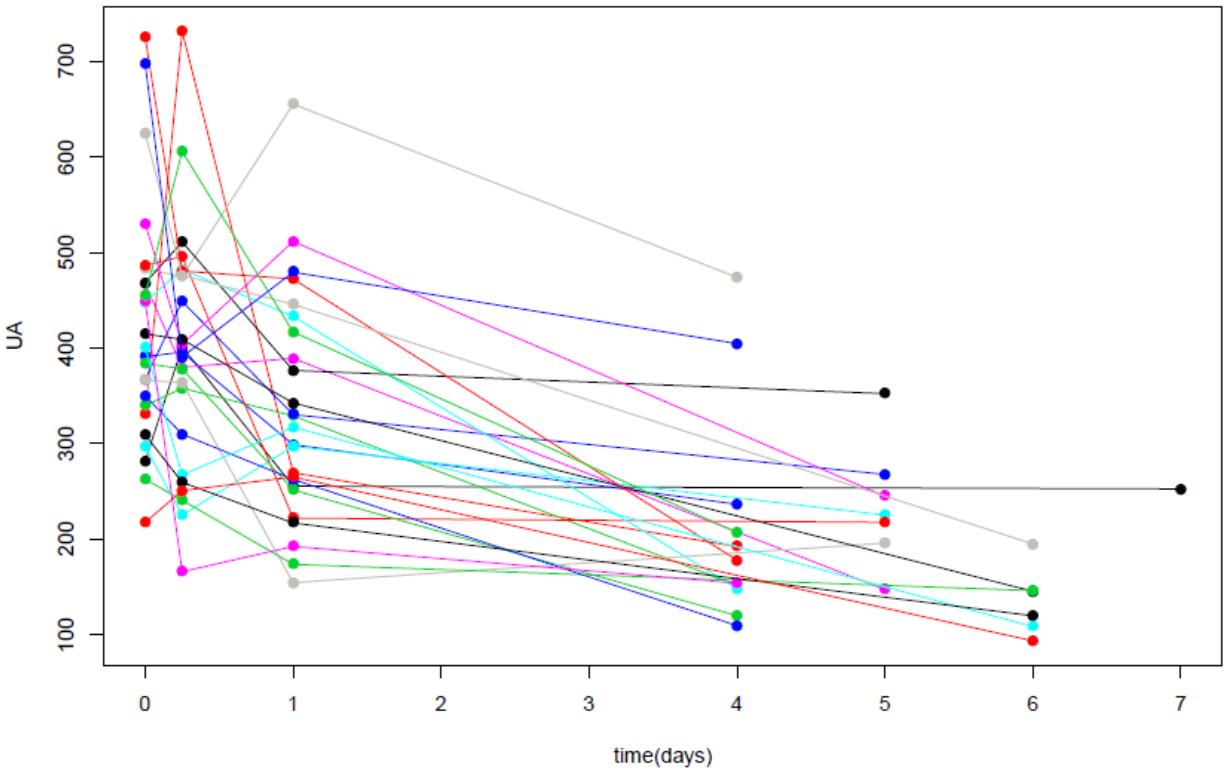


Figure 12 Uric acid levels at high dose

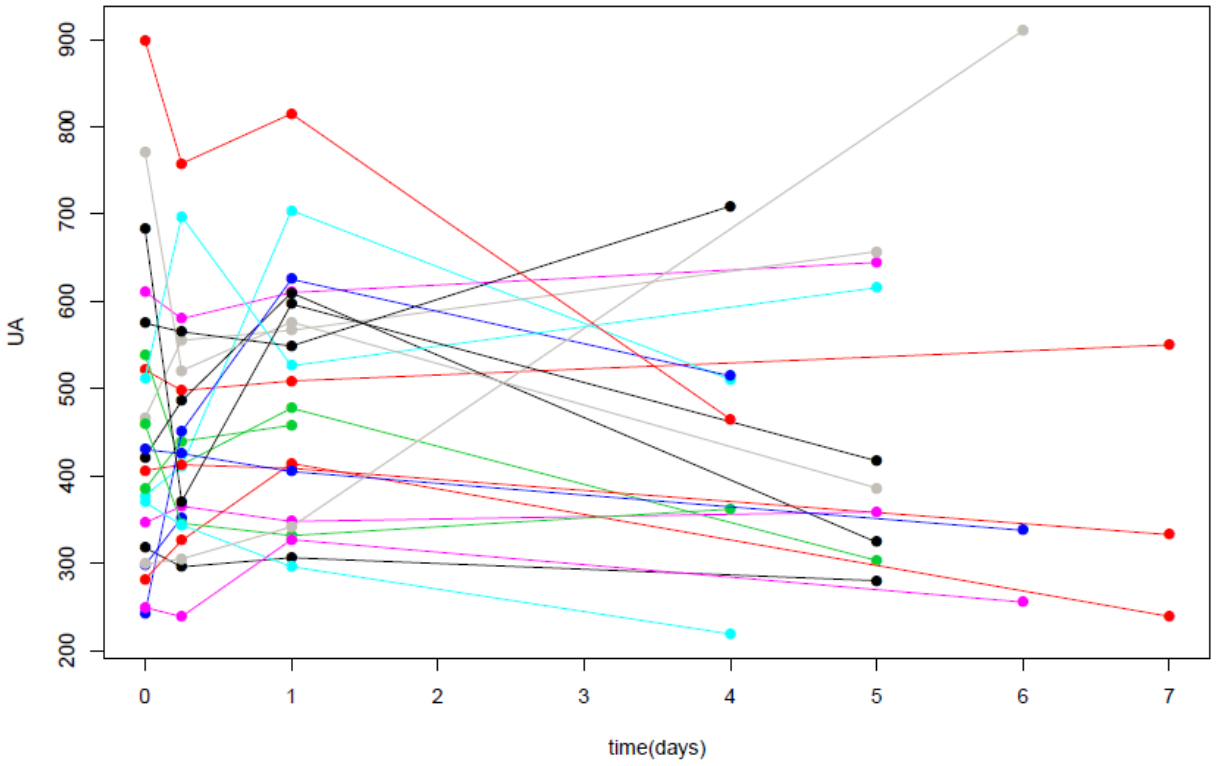


Figure 13 Uric acid levels placebo

A a

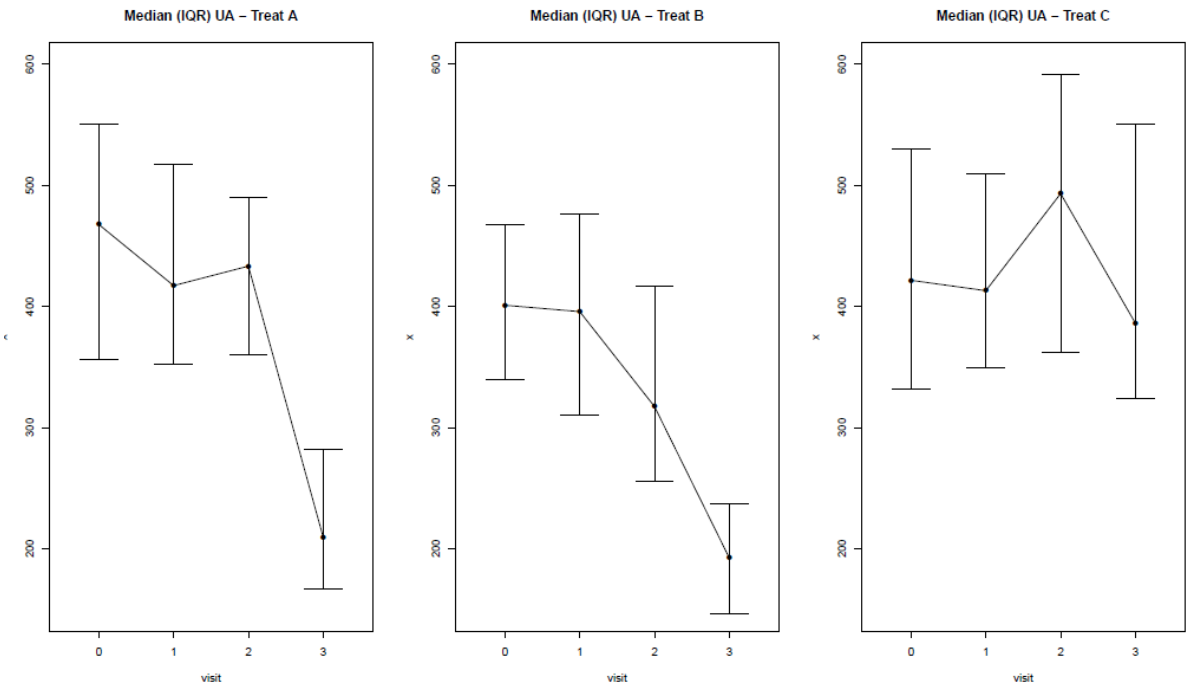


Figure 14 median uric acid per treatment arm

Treatment A is low dose allopurinol, B high dose allopurinol and C placebo. The median plots also show that patients were compliant with their medications.

3.4 Adverse Events

One patient had a serious adverse event when he became very unwell an hour after the loading dose in the second arm. He had an episode of collapse with loss of consciousness and hypotension but no ECG changes, followed by a second collapse episode. He was admitted to hospital for an overnight stay and all investigations were normal. The cause for his collapse episodes were not clear. This was reported and fully investigated by the monitoring committee. The patient was withdrawn from the study.

Another patient developed a rash during the wash out period between the second and third arm, thought to be unrelated to the study.

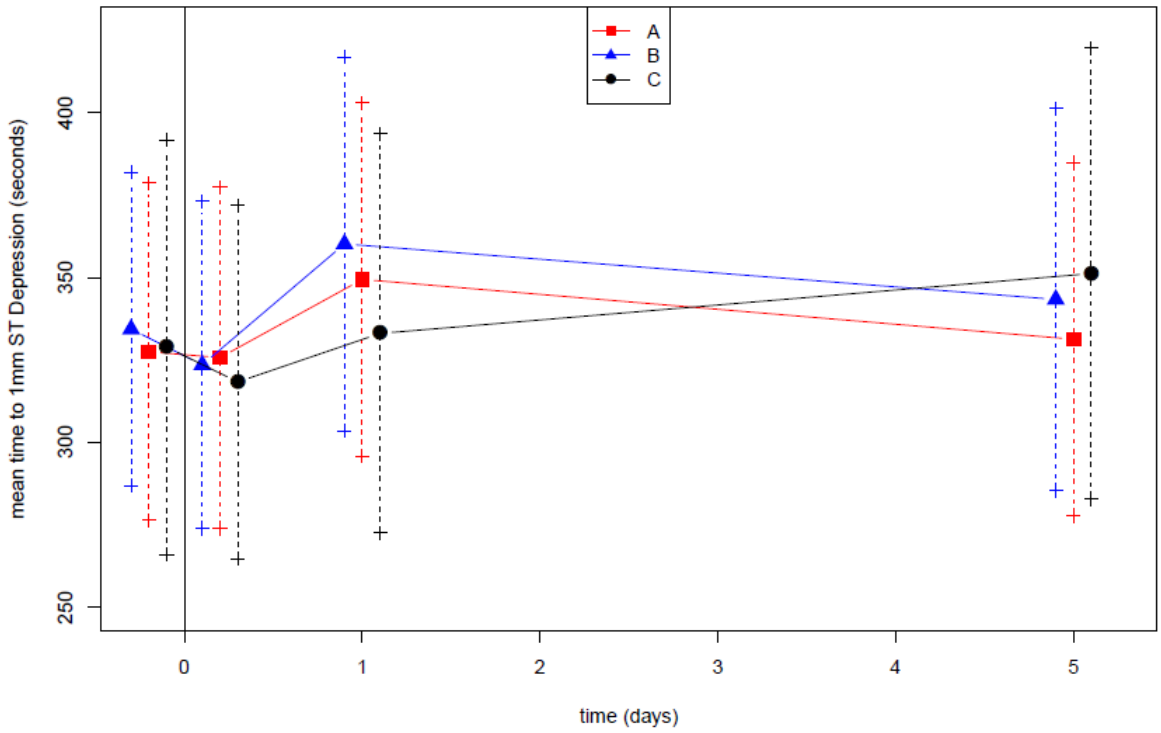
3.5 Exercise treadmill test

The exercise treadmill results were independently validated by a blinded reviewer and were consistent in 98%. Discrepancies were reviewed and final data agreed.

The output from Open Clinica provided the time to ST depression, the total exercise time and the time to chest pain for each ETT for each subject. For each of the time to ST depression and total exercise time, a linear mixed model was fitted using the SAS 9.3 PROC MIXED procedure, including the baseline measurement, treatment (A, B, C), period (1,2,3), measurement replication number (1,2,3) and a period-by-treatment term and with a compound symmetry correlation structure between repeated measures between person and treatment. [87] This type of analysis was recommended by the statistician for the trial.

3.5.1 Time to ST depression (st1mm)

The figure below shows the mean time to ST depression for each treatment arm. A is low dose allopurinol, B is high dose allopurinol and C is placebo. This shows that the time to ST depression was higher in the allopurinol arms although this was not significant.



A low dose, B high dose, C placebo

Figure 15 Mean time to ST depression for each arm

Spaghetti plots were then done for each subject in each arm of the trial to look for obvious outliers.

Figures 16-18 show these:

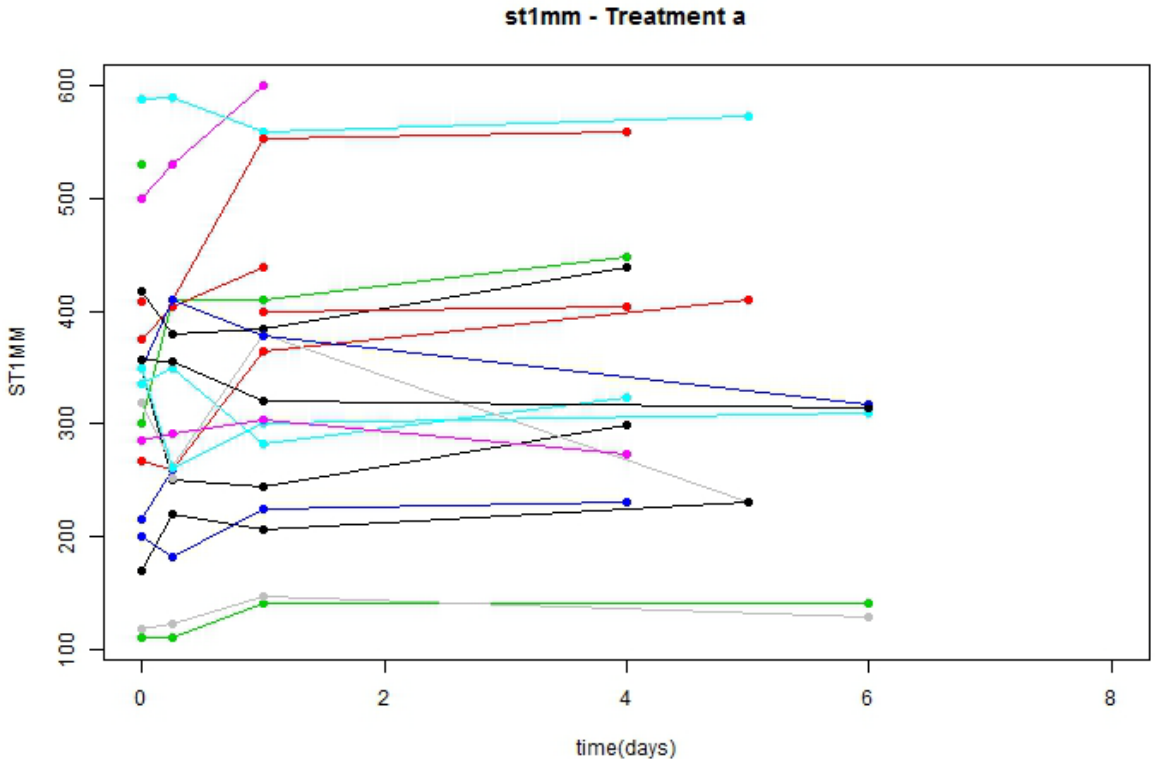


Figure 16 Time to ST depression low dose allopurinol

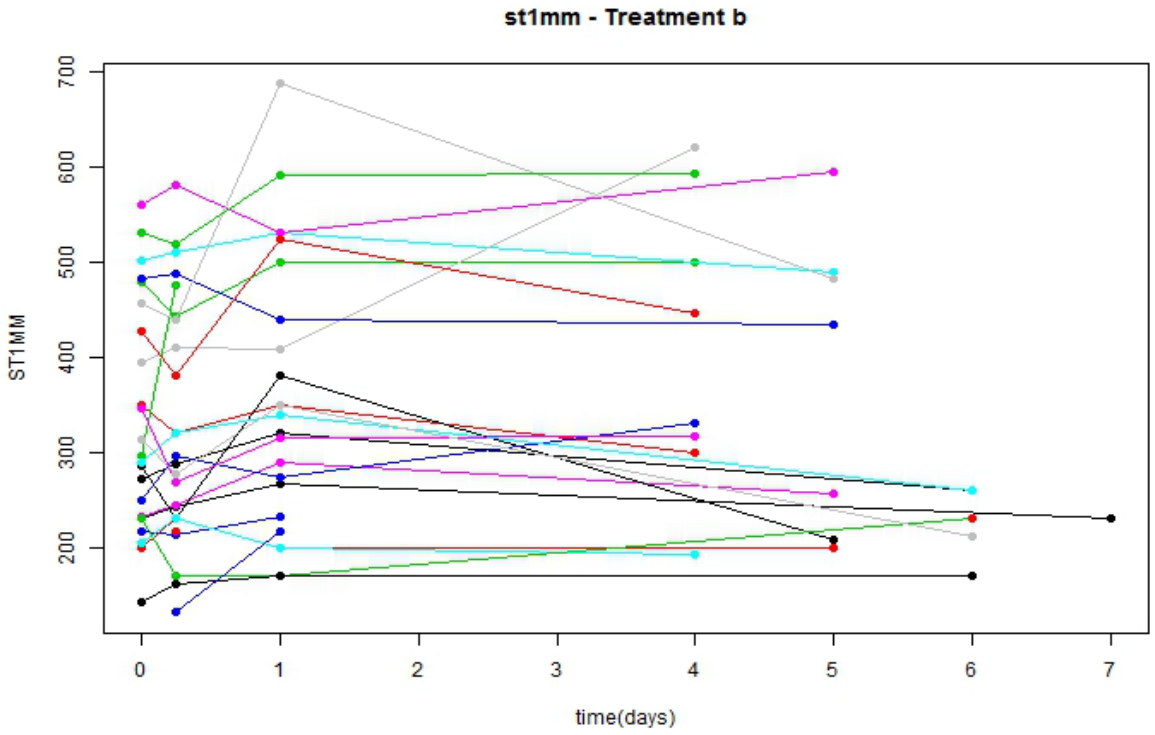


Figure 17 Time to ST depression high dose allopurinol

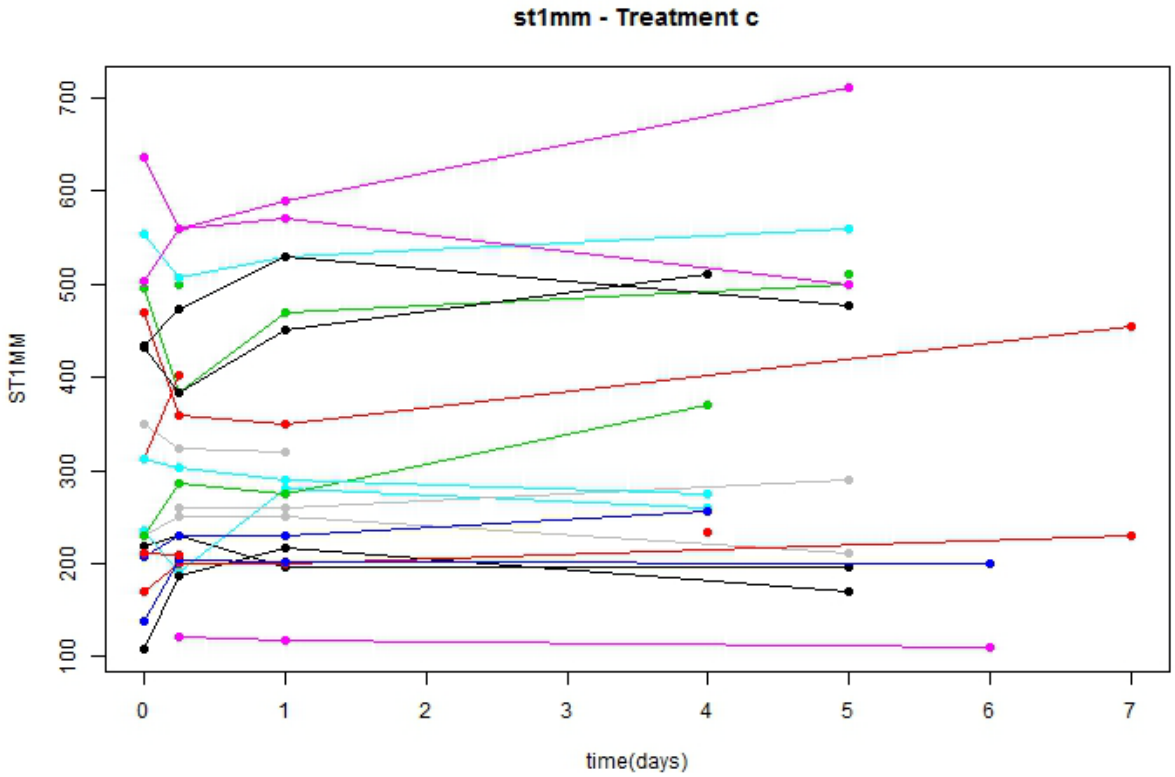
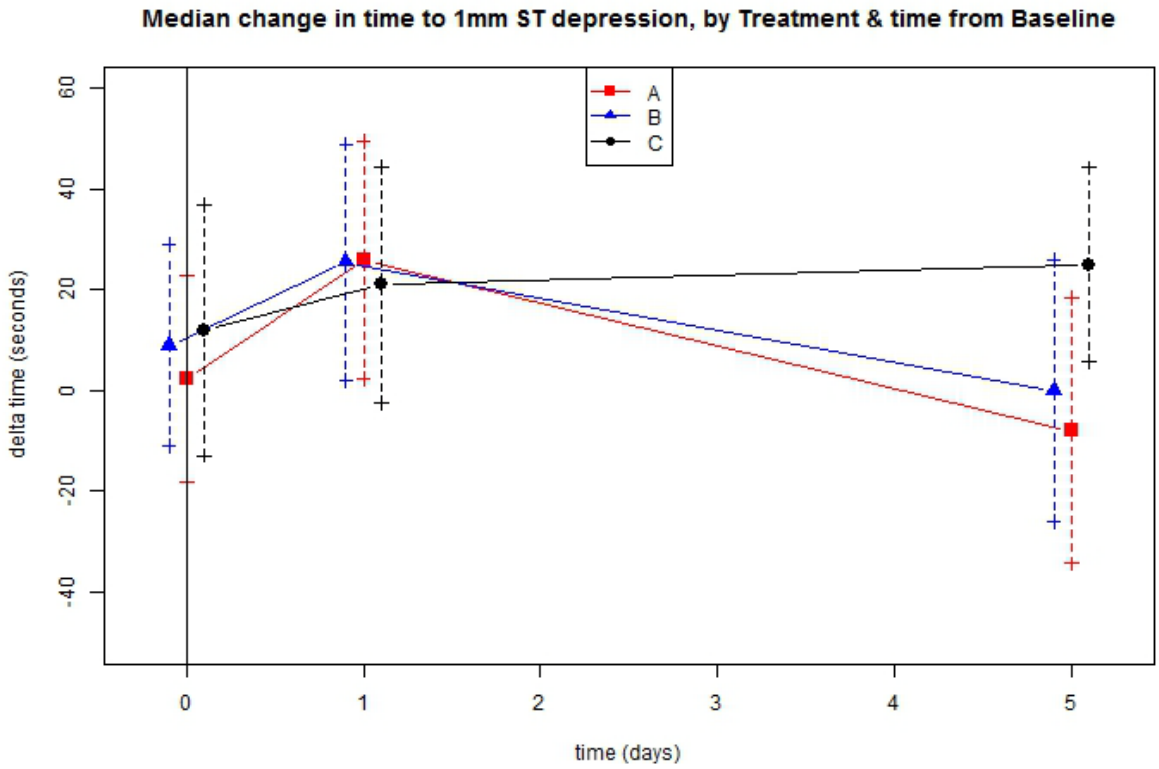


Figure 18 Time to ST depression placebo

The median difference was also plotted as shown in figure:



A low dose, B high dose, C placebo

Figure 19 Median change in time to ST depression

The baseline (pre-dose) measurement had the biggest effect on post dose measurement (P<0.0001) but no significant overall treatment effect on time to ST depression (P=0.78)

Overall there was no treatment difference on time to ST depression.

- A - B: difference (95% CI) = -6.4 (-25.2,12.4), p=0.49
- A - C: difference (95% CI) = -4.7 (-23.9, 14.5), p=0.62

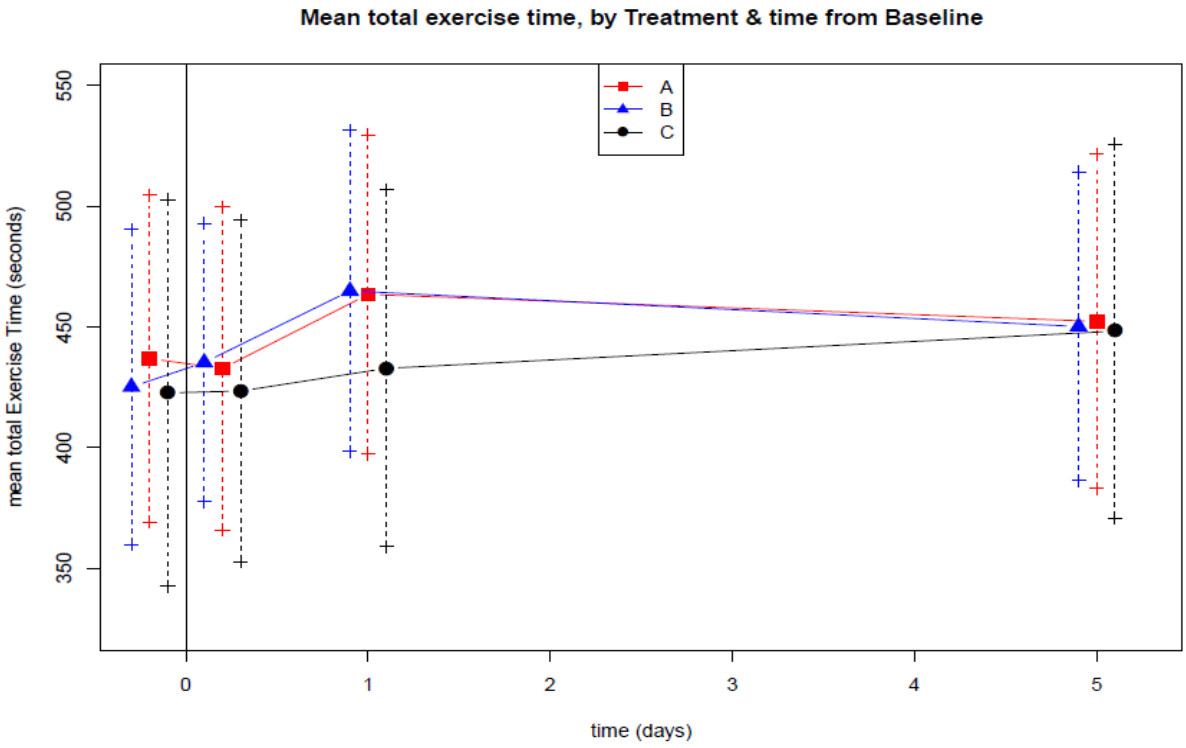
- B - C: difference (95% CI) = 1.7 (-17.1, 20.6), p=0.85

  A is low dose, B is high dose and C is placebo.

  The P values and the fact the confidence intervals cross 0 both confirm there was no significant effect.

3.5.2 Total exercise time

The figure below shows the mean total exercise time for each treatment arm. A is low dose allopurinol, B is high dose allopurinol and C is placebo. This shows that the total exercise time was higher in the allopurinol arms at 24 hours.



A low dose, B high dose, C placebo

Figure 20 Mean total exercise time by treatment and time from baseline

There was significant overall treatment effect on total exercise time (P=0.024)

This showed up in the effect of treatment differences, with a significant effect of both treatments A & C compared to B on total Exercise time.

- A - B: difference (95% CI) = -18.0 (-33.9,-2.0), p=0.028
- A - C: difference (95% CI) = 3.1 (-13.4, 19.5), p=0.71
- B - C: difference (95% CI) = 21.0 (4.8, 37.3), p=0.012

This shows that high dose (B) was more effective than low dose (A) and placebo (C). Low dose (A) was also more effective than placebo (C) although this difference was not significant as P=0.71 and the confidence intervals cross 0.

The Figures below show the spaghetti plots for each subject in each arm of the trial.

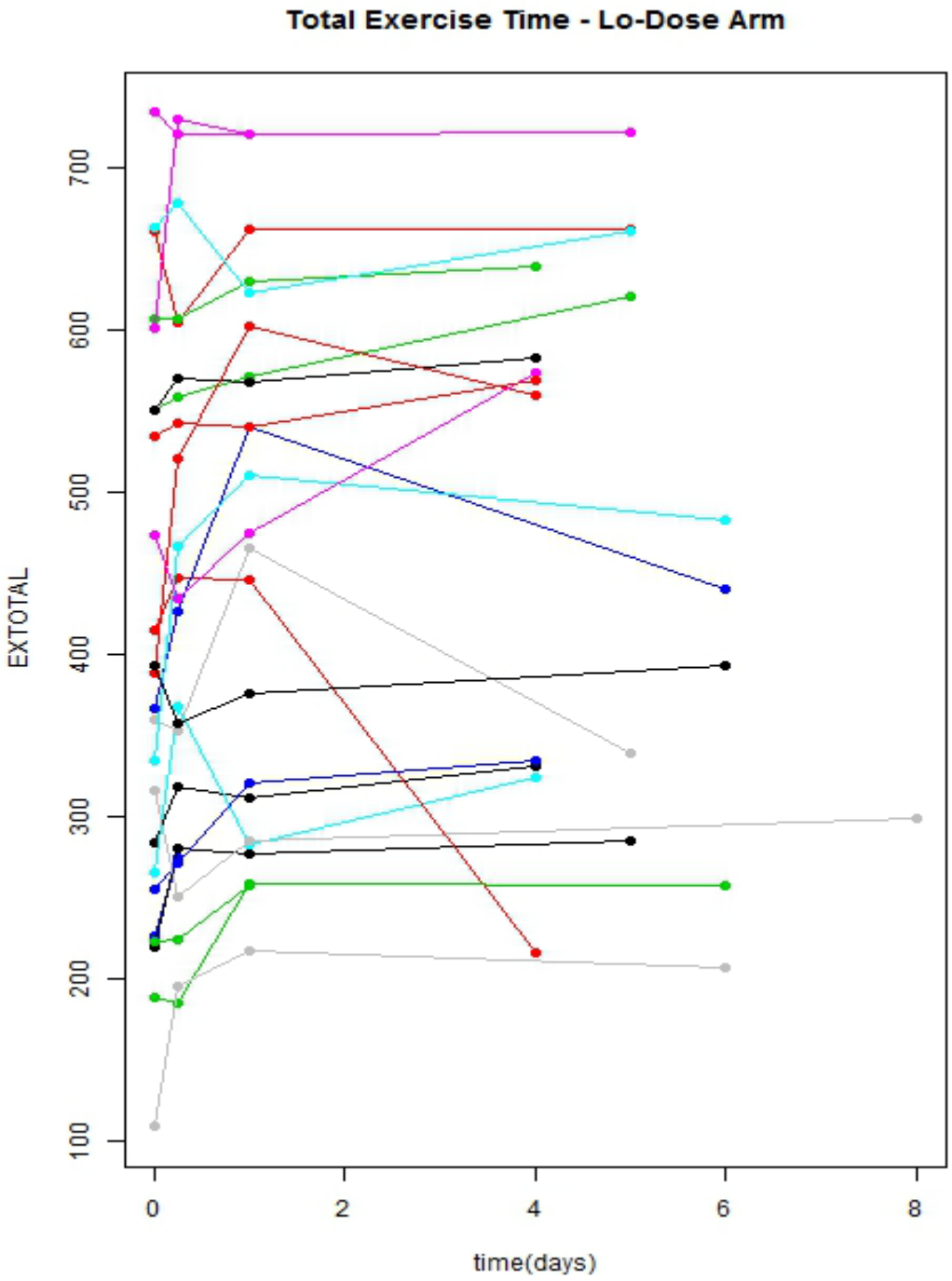


Figure 21 Total exercise time low dose allopurinol

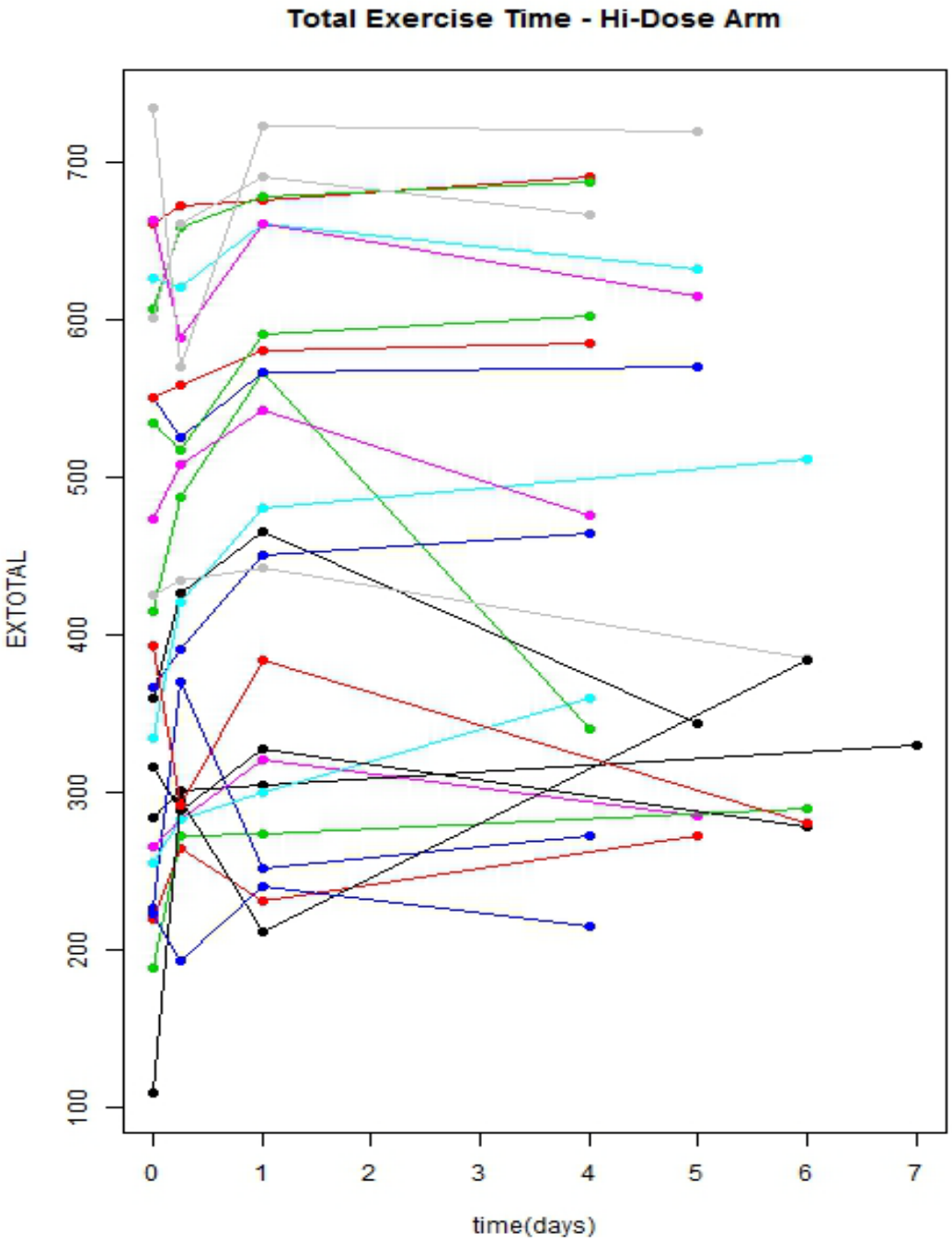


Figure 22 Total exercise time high dose allopurinol

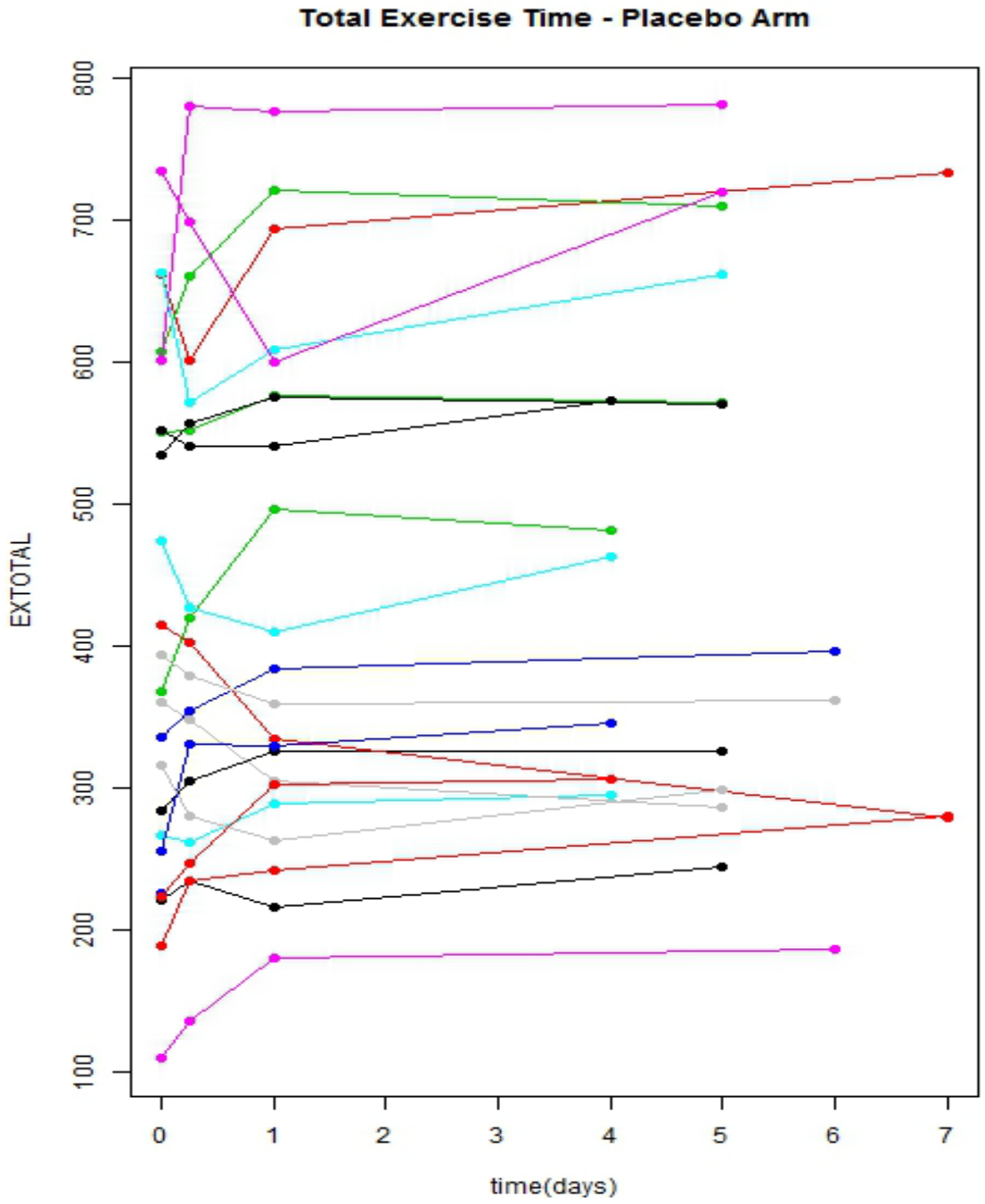


Figure 23 Total exercise time placebo

Each subject's exercise time progression throughout the trial was then plotted. (Figure 24)

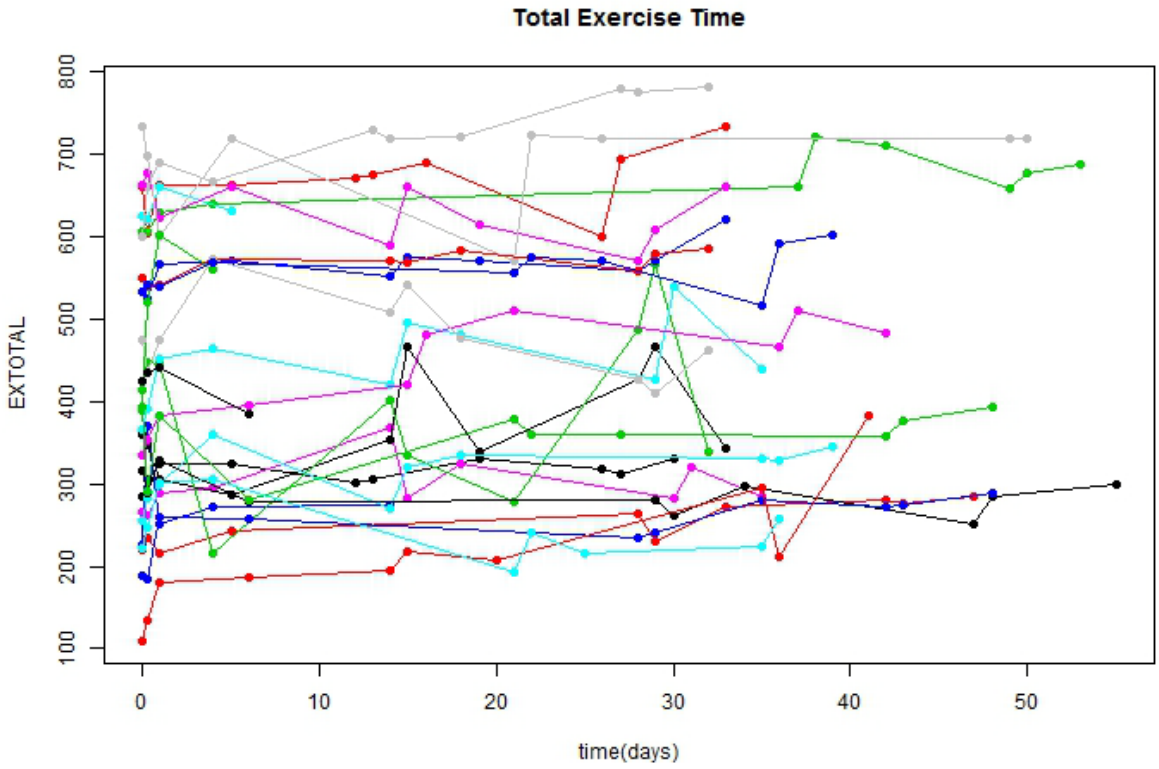


Figure 24 total exercise time for each subject across study

It was felt there was general trend to increase in total exercise time from start to finish so this was analysed further by looking at the total exercise time changes in each arm compared to the baseline exercise time.

The figures below show the range of total exercise time at baseline and final ETT for each arm, with the third box plot showing the difference in time from baseline to final ETT. The figures represent the placebo arm, the low dose arm and the high dose arm consecutively.

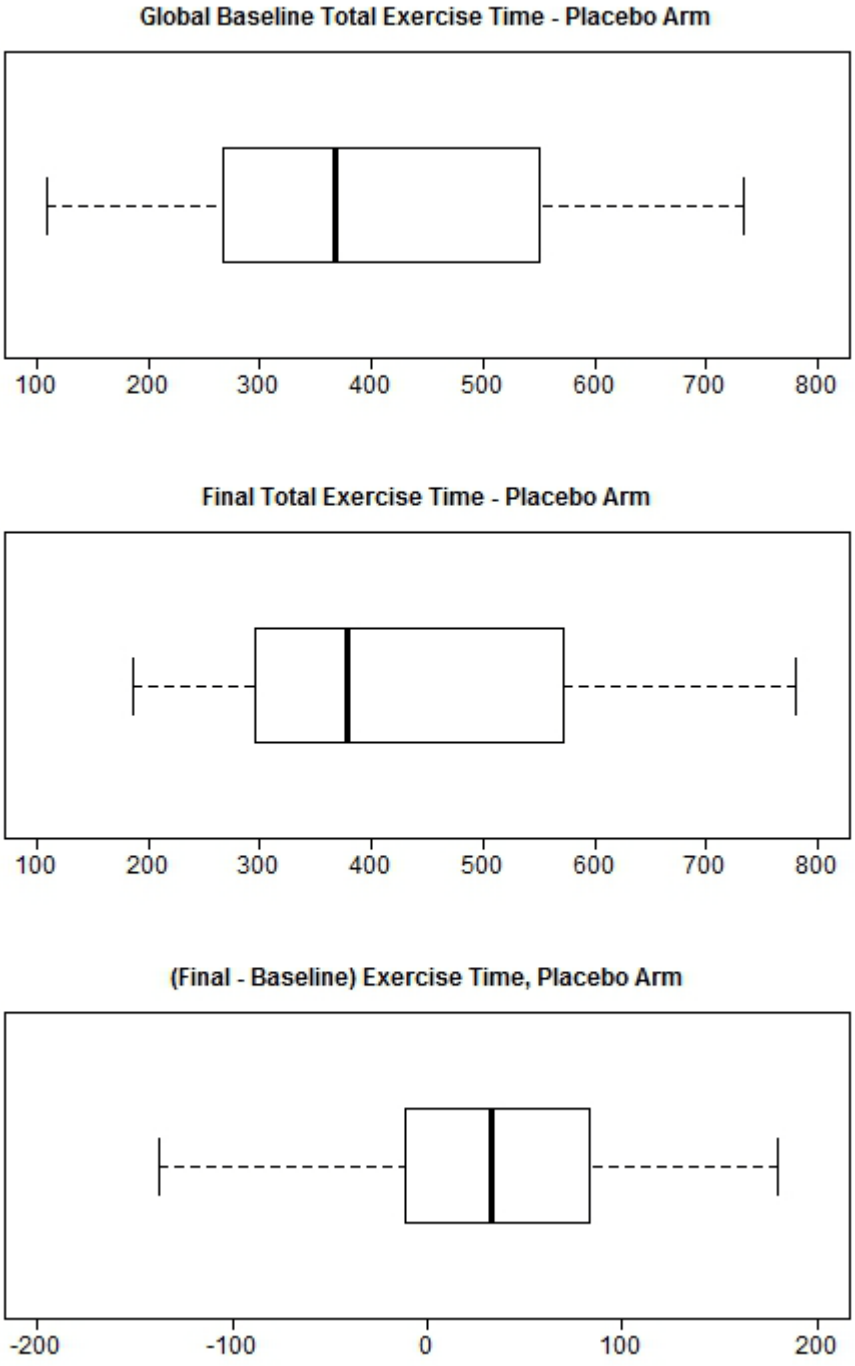


Figure 25 Boxplots of total exercise time placebo arm

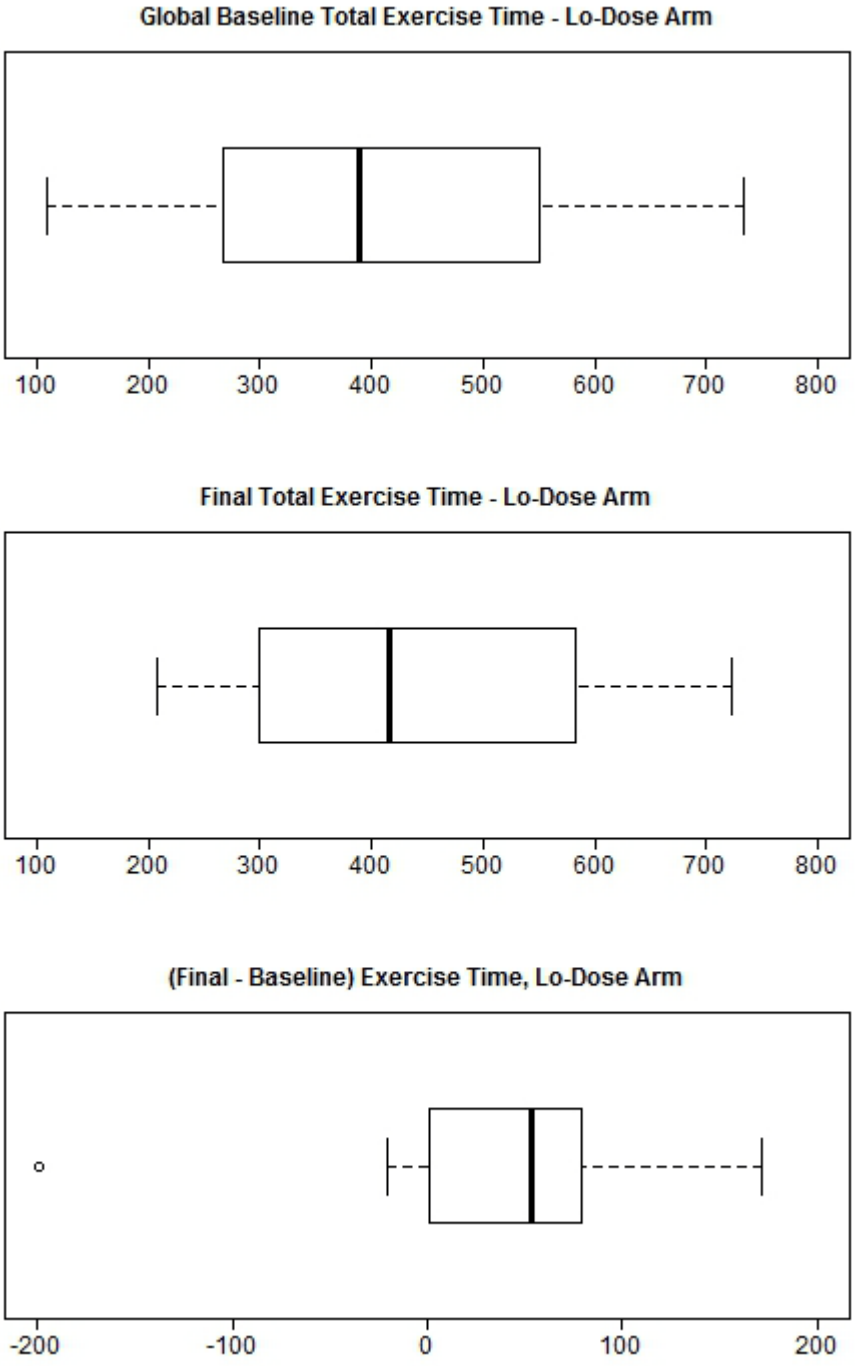


Figure 26 Box plots of total exercise time low dose arm

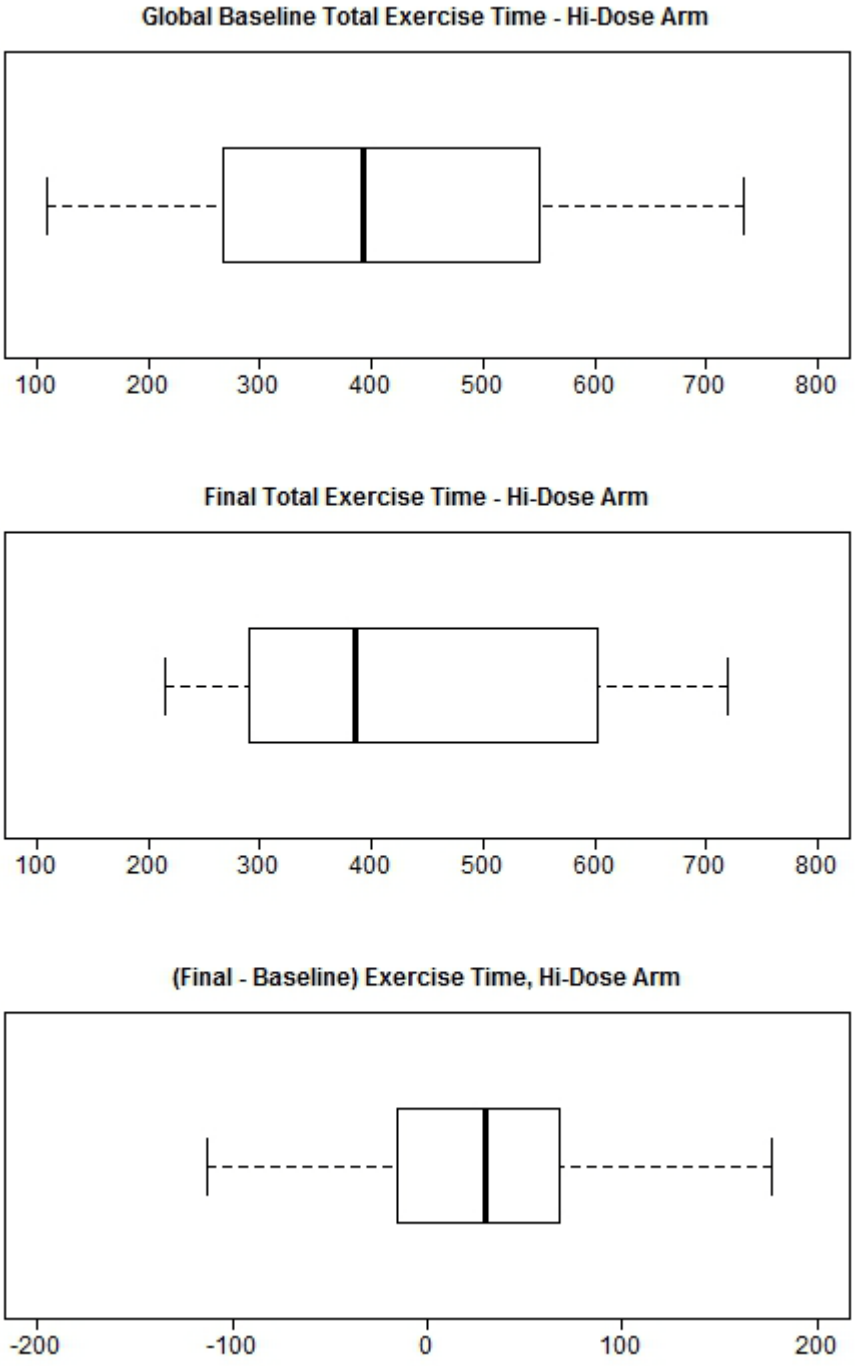


Figure 27 Boxplots of total exercise time high dose arm

The numbers are detailed in the table below.

		Placebo (c)	High-dose (b)	Low-Dose (a)
Global Baseline	Min	109	109	109
	Q1	266	266	266
	Q2	**367**	**393**	**389**
	Q3	551	551	551
	Max	734	734	734
	Mean (sd)	**411 (174)**	**415 (173)**	**413 (170)**
Final visit within arm	Min	186	215	207
	Q1	295	290	299
	Q2	**378.5**	**385**	**416.5**
	Q3	572	602	583
	Max	781	719	722
	Mean (sd)	**448 (183)**	**450 (161)**	**444 (164)**
Final - BL	Min	-137	-113	-199
	Q1	-11	-15	1
	Q2	**32.5**	**30**	**53.5**
	Q3	83	68	79
	Max	180	275	171
	Mean (sd)	**34 (68)**	**35 (80)**	**45 (73)**
	t-test (final - BL)	t=4.12, p=0.00011	t=3.8, p=0.00031	t=5.04, p<1e-05
	Wilcox test (final – BL)	V=1746, p=0.000043	V=2082, p=0.00053	V=1758, p<1e-06

Table 12 Total exercise time changes over global baseline by arm

The global baseline is the total exercise time at the first visit of each arm, the final exercise time is the total exercise time on the final ETT at the end of each arm and the difference between these times is shown at the bottom. Q2 represents the median value. The paired t-test uses the individual paired (baseline and final) values to see if the mean of

baseline and mean of final values were different and found there was a significance difference in each arm. To allow for the possibility of the data not being normal distributed wilcox tests performed using the median valves.

3.5.3 Time to chest pain during exercise

As the patients did not regularly get chest pain on the treadmill there was not a reliable amount of data to analyse from this perspective. In total nine patients reported pain at baseline during the study in one or more of the arms with reporting pain during the high dose arm, seven during the low dose arm and eight reporting chest pain during the placebo arm.

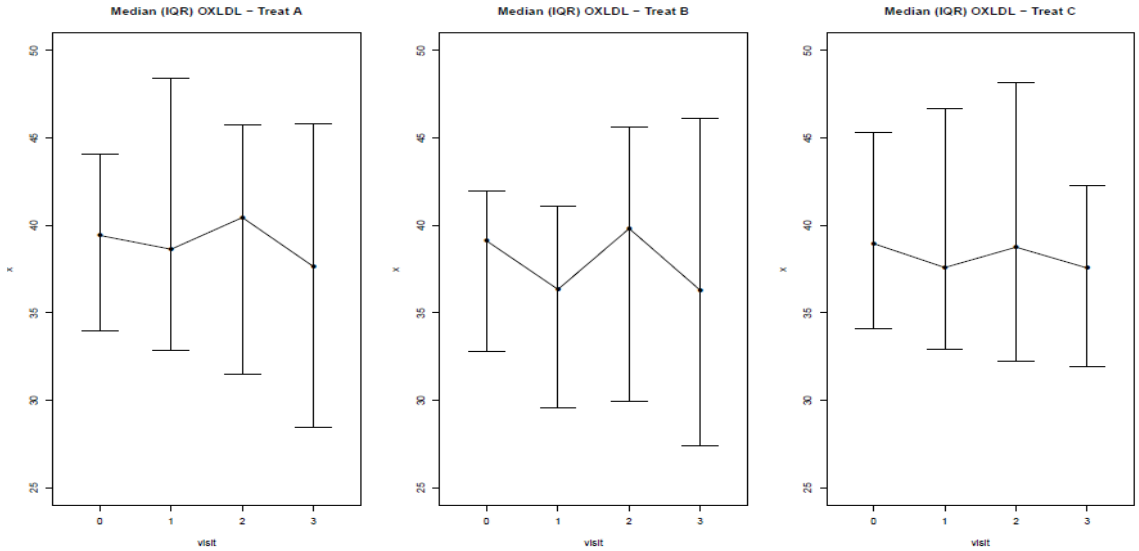
3.6 Blood tests

3.6.1 Troponin

Troponins were not detected in any of the patients throughout this study.

3.6.2 Oxidised LDL

Figure shows the median oxidised LDL for each arm of the trial and visit. Treatment A is half dose, B full dose and C placebo. Visit 0 refers to the baseline blood sample before the trial drug was given, visit 1 refers to the post ETT bloods taken at the 4 hour ETT, visit 2 refers to the post ETT bloods at 24 hours and visit 3 to the post ETT bloods taken on the final visit of the arm.



A low dose, B high dose, C placebo

Figure 28 Median Oxidised LDL for each treatment arm

Figures 30-32 show the oxidised LDL level for each subject in each arm of the trial.

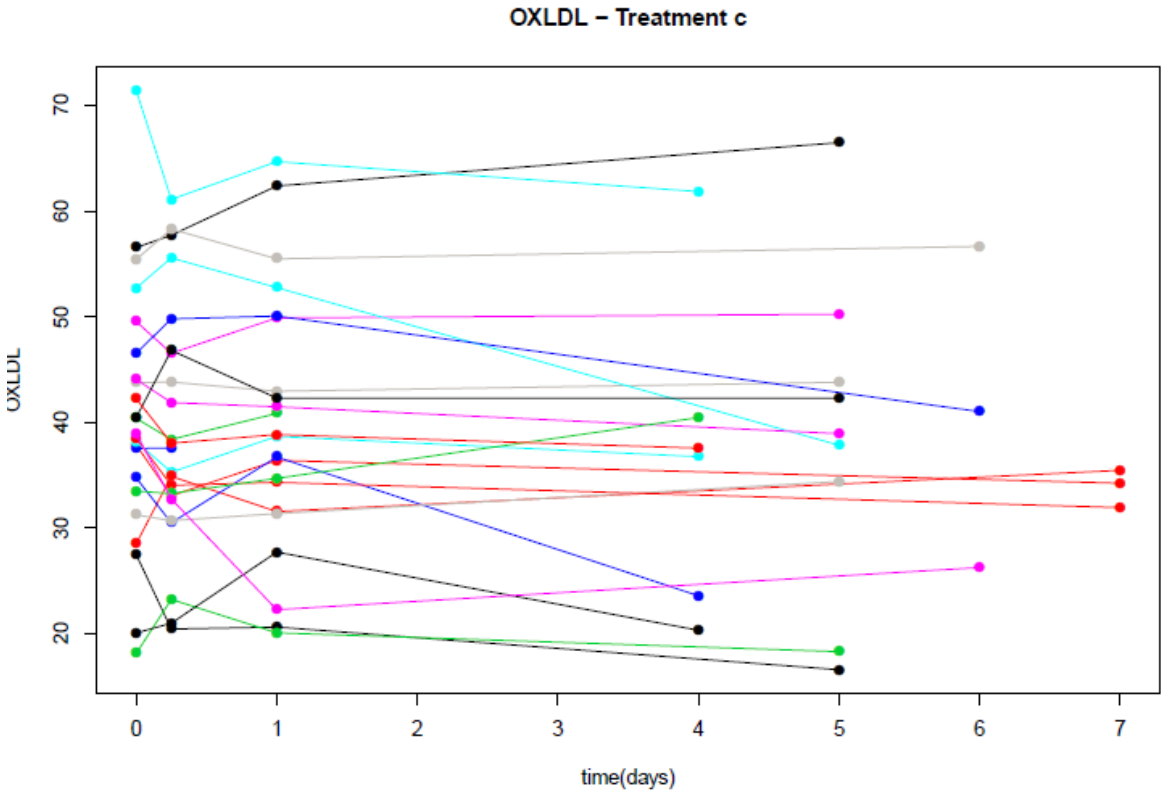


Figure 29 Oxidised LDL per subject placebo

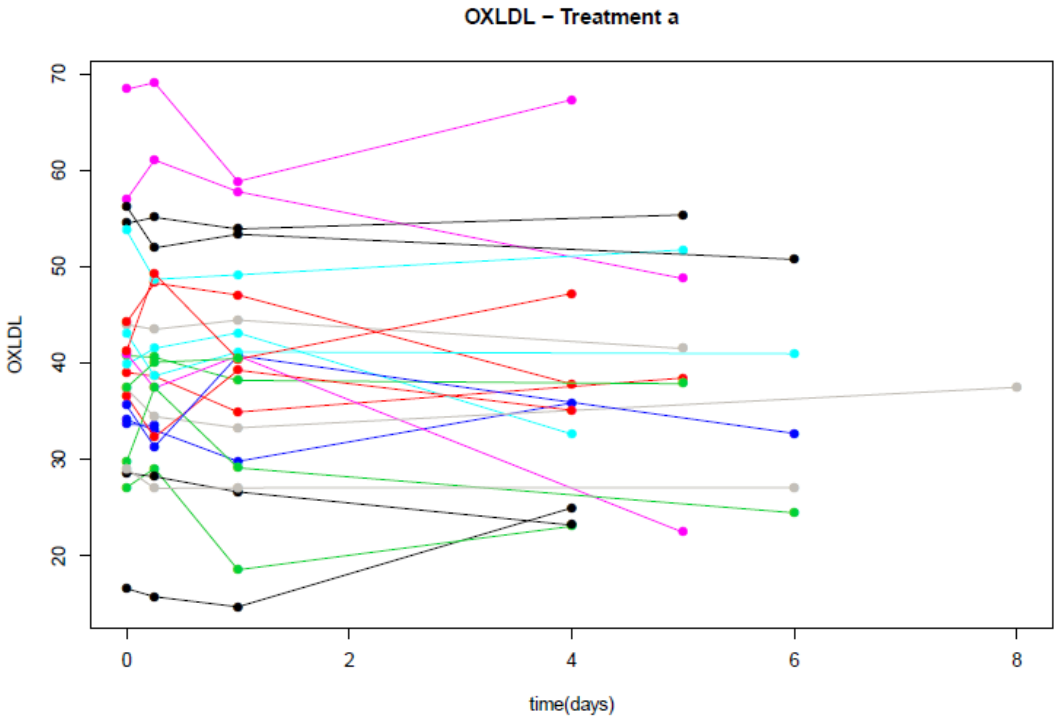


Figure 30 Oxidised LDL per subject low dose allopurinol

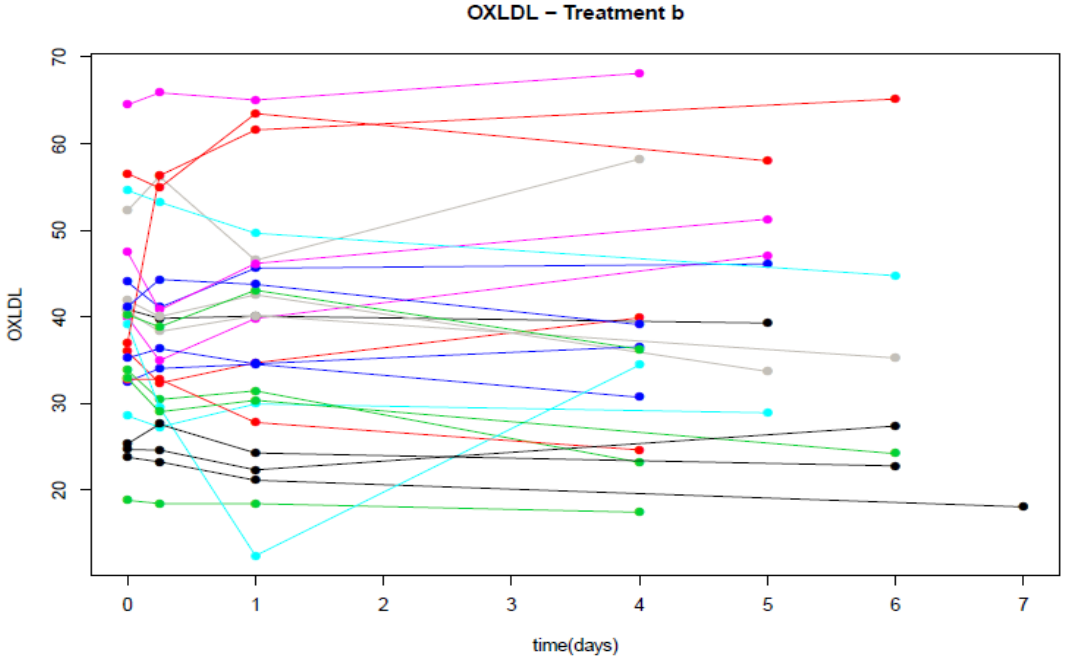
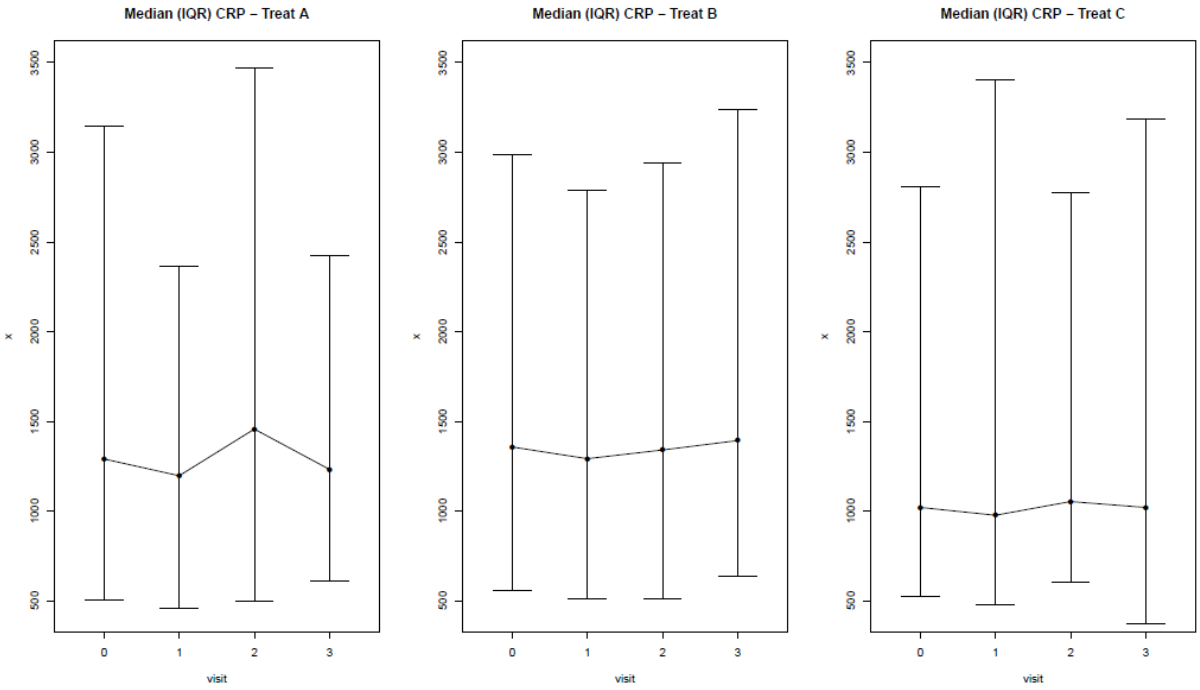


Figure 31 Oxidised LDL per subject high dose allopurinol

3.6.3 CRP

Figure 32 shows the median CRP for each arm of the trial and each visit. Treatment A is low dose, treatment B is high dose and C is placebo. Visit 0 refers to the baseline blood sample before the trial drug was given, visit 1 refers to the post ETT bloods taken at the 4 hour ETT, visit 2 refers to the post ETT bloods at 24 hours and visit 3 to the post ETT bloods taken on the final visit of the arm.



A low dose, B high dose, C placebo

Figure 32 Median CRP for each treatment arm

Figures 33-35 show the CRP for each subject in each arm of the trial.

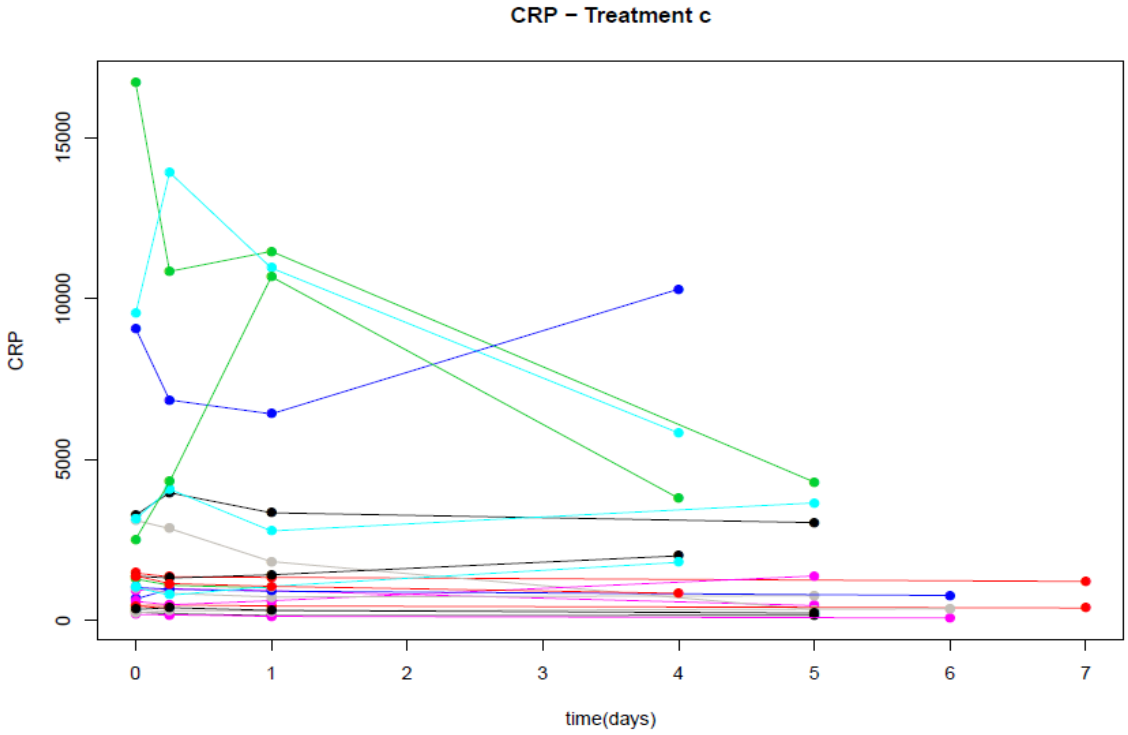


Figure 33 CRP per subject placebo

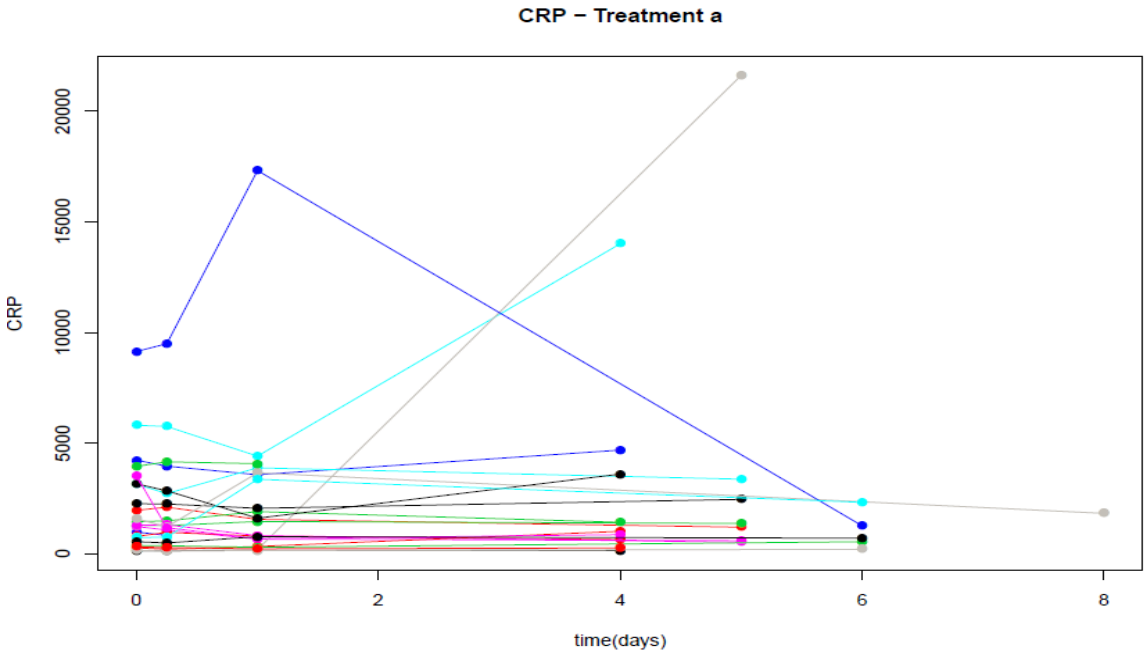


Figure 34 CRP per subject low dose allopurinol

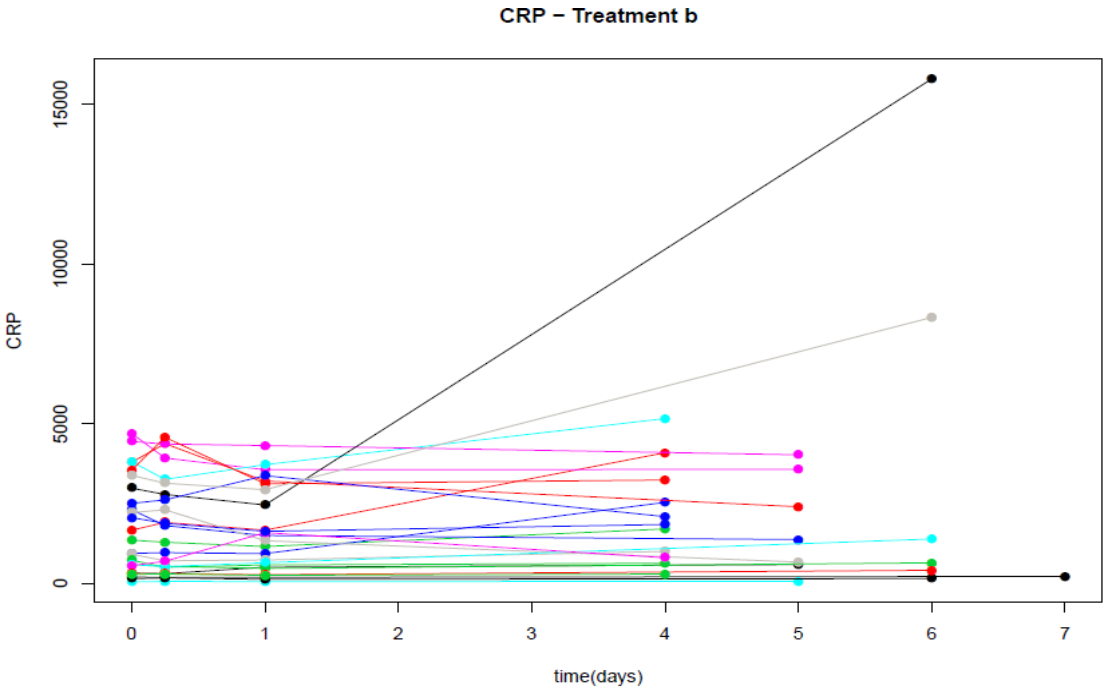
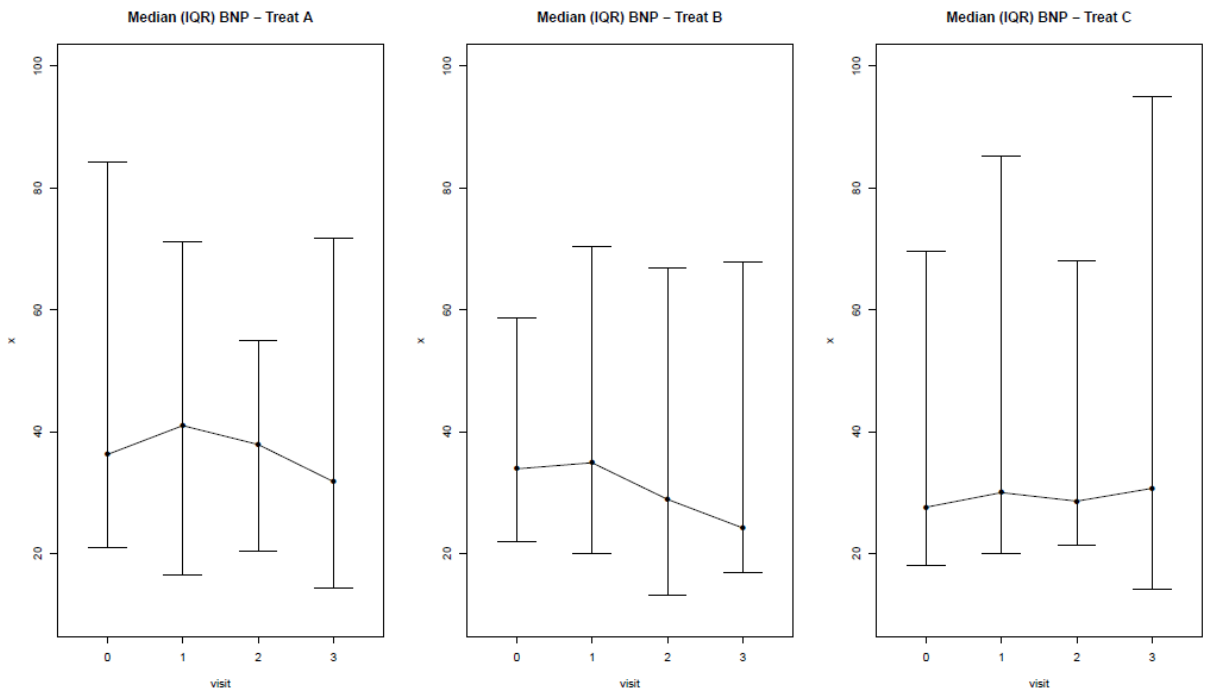


Figure 35 CRP per subject high dose allopurinol

3.6.4 BNP

Figure 36 shows the median BNP for each arm of the trial and each visit. Treatment A is low dose, treatment B is high dose and C is placebo. Visit 0 refers to the baseline blood sample before the trial drug was given, visit 1 refers to the post ETT bloods taken at the 4 hour ETT, visit 2 refers to the post ETT bloods at 24 hours and visit 3 to the post ETT bloods taken on the final visit of the arm.



A low dose, B high dose, C placebo

Figure 36 Median BNP per arm

Figures 37-39 show the BNP for each subject in each arm.

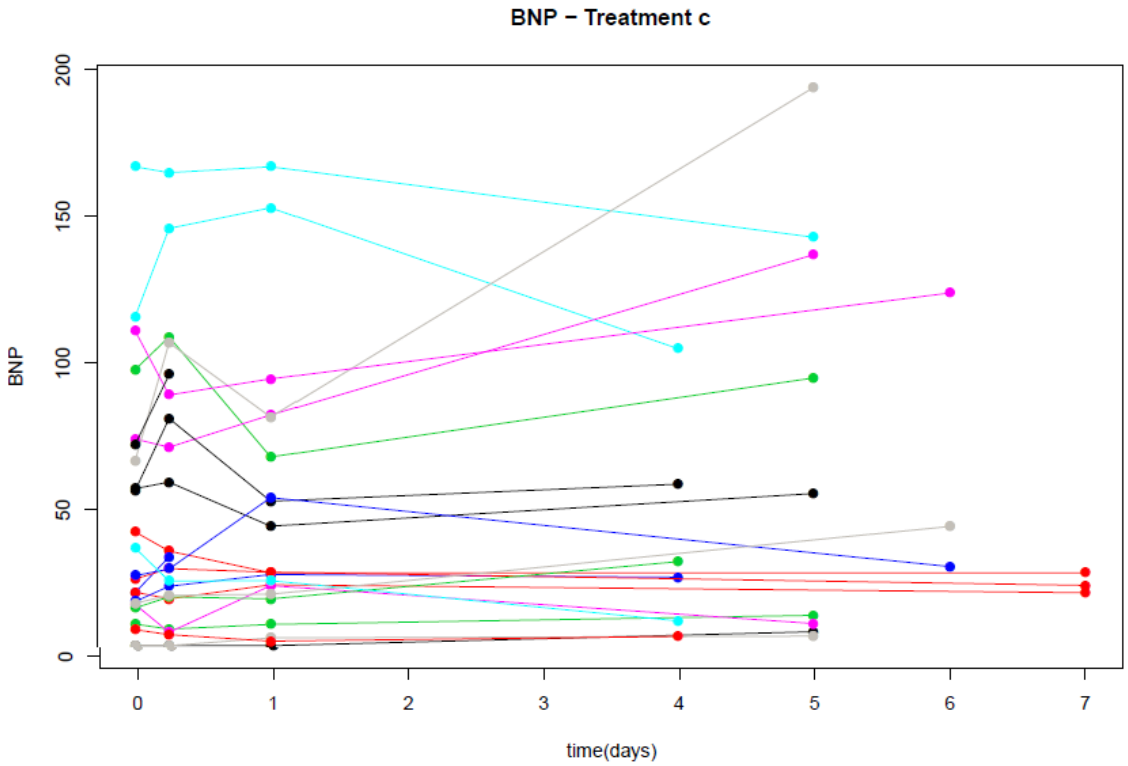


Figure 37 BNP per subject placebo

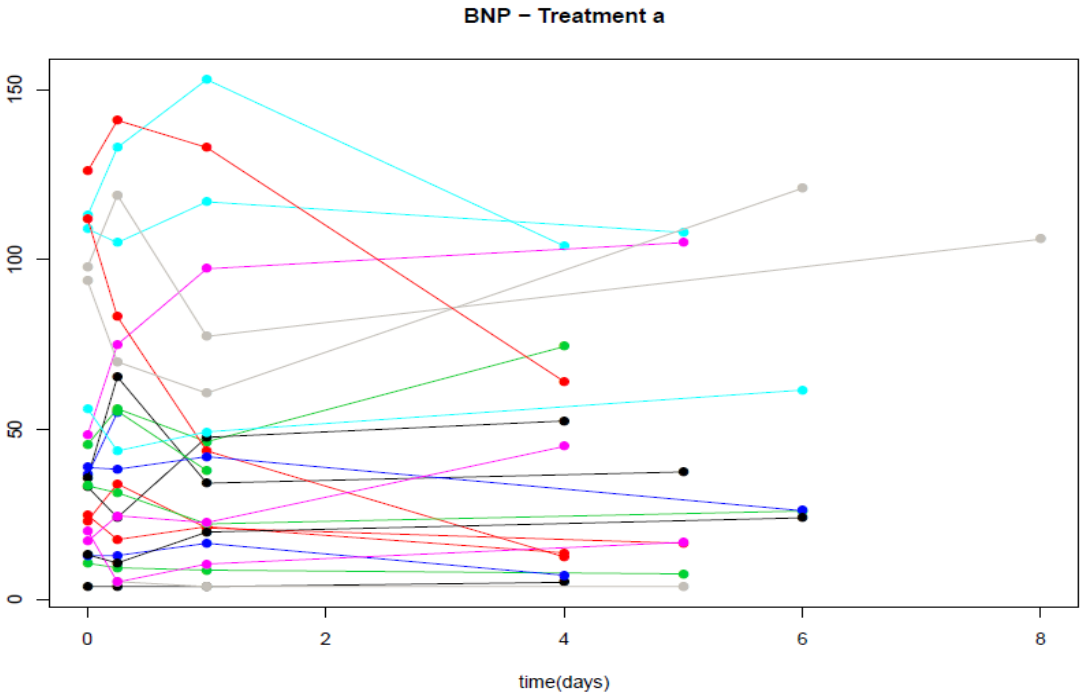


Figure 38 BNP per subject low dose allopurinol

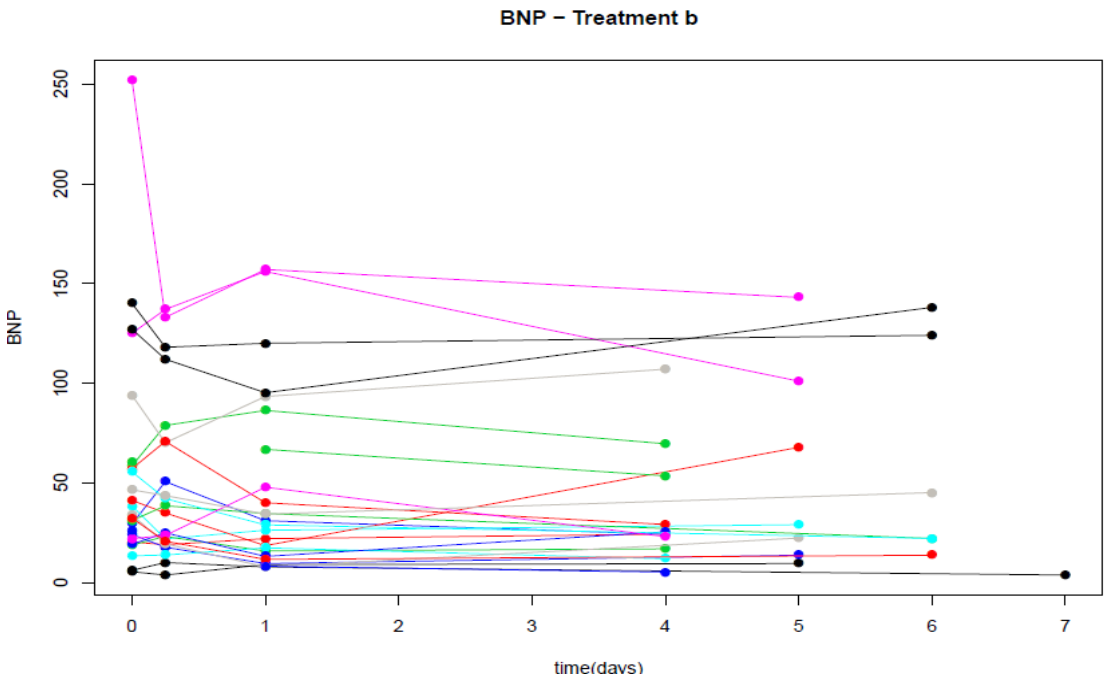


Figure 39 BNP per subject high dose allopurinol

3.7 Angina Diary

The return of diaries was insufficient to allow any meaningful analysis. This was due to the poor completion of the diaries and infrequency of chest pain experienced by patients. Since each treatment phase lasted such a short time it was never anticipated that the diary data would be meaningful.

4 Discussion

This double-blind, placebo controlled, three arm cross over trial aimed to determine the time of onset and the optimal dose of allopurinol in patients with coronary artery disease using patients with stable disease as a surrogate for acute coronary syndromes. In this section the findings of the study (already presented in the results section) will be discussed and the possible underlying mechanisms for these findings, along with the strengths and limitations of this study, will be considered.

4.1 Recruitment

Recruitment for this study proved to be extremely challenging. This was in essence due to a local change in clinical practice. Patients had previously undergone diagnostic angiograms in Tayside and were referred across to Edinburgh Royal Infirmary if they required intervention. This meant patients with coronary artery disease could be identified and recruited to participate in clinical trials while they awaited being listed for interventional procedures. Now patients undergo one procedure whereby coronary artery disease is identified and treated during the same procedure.

Significant effort went into recruiting patients and many avenues were explored in an effort to identify patients. This included extending to using the Perth Royal Infirmary exercise treadmill, extending to Fife to recruit more patients and exploring extending to Grampian.

This study also required a significant commitment from patients who had to be available three days within one week and furthermore had to be able to repeat this commitment twice to complete the study. There was also a high intensity of exercise with four treadmills within one week which demanded a degree of fitness from the patients. Despite a

high number of patients being invited to participate in the trial the uptake was low. Furthermore the changes in clinical practice, which means symptomatic patients are stented much earlier, meant that many patients did not have ongoing ischaemia required to produce ST changes on treadmill test.

The challenges of recruitment within this study are a cautionary tale which highlight the importance of resilience in trial design to adapt to the often rapid changes in clinical practice.

4.2 Primary Outcome – Time to ST depression on ETT

The results of the primary outcome are laid out in section 3.5.1. It can be seen that the mean time to ST depression was shorter in all arms of the trial on the ETT four hours post loading. This may be because this was the second ETT of the day for the patients or it may indicate allopurinol does not work within this short time frame. Interestingly by 24 hours post loading, for both the low dose and high dose arms of the trial there was an increase in the time to ST depression. This was not sustained by day 5 in either active arms of the trial. Furthermore in the placebo arm of the trial the total time to ST depression showed a gradual increase over the arm. None of these changes in time to ST depression shows statistical significance but this may be due to the fact the study was underpowered due to small number of participants. It is also worth noting the median uric acid levels did not fall until the third visit of each arm which would suggest the biological effects are mainly after the study period ends. This may explain why the previously noted effects of allopurinol on time to ST depression are not shown in this study.[44] It is also possible the drug effects on tissue oxidative stress described in the introduction, are exerted before the drug reduces the

urate level which is why the time to ST depression initially increased although it is difficult to explain why this effect would then be lost.

Interestingly if we look at the spaghetti plots for each individual subject there are some outliers for whom the time to ST depression does increase in each of the active arms of the trial but not in the placebo arm.

4.3 Secondary Outcomes

4.3.1 Total exercise time

It was also interesting to note that as per the primary outcome, at 24 hours there appears to be improvement in the mean exercise time in both treatment arms which is not fully sustained on day 5. In addition the placebo arm shows a progressive increase in total exercise time across the arm and the final total exercise time for all three arms are very similar. In the high dose arm the total exercise time fell four hours after the loading dose, but increased slightly in the low dose and the placebo arm. In view of the delay in fall of uric acid noted above it is difficult to explain these observations. Was the improvement noted at 24 hours simply chance? This seems unlikely based on the previous work by Noman et al.[44] However, as the sub-analysis of the total exercise time revealed, there was a significant improvement in all arms of the trial in terms of total exercise time which may reflect a degree of conditioning. This may suggest the five day negative effect was due to a the training effect overwhelming any drug effect. The effects of high intensity training in cardiovascular disease is discussed below.

4.3.2 Time to chest pain

As patients did not reliably experience chest pain during ETT there was insufficient data to draw any conclusions. Patients also did not complete their chest pain diaries. However the infrequency of chest pain experienced, again reflects the changes in clinical practice in that patients with ongoing chest pain undergo coronary intervention to alleviate their symptoms if these are not controlled medically. NICE guidelines [88] state that if patients symptoms are not controlled with two anti-anginal drugs, they should only be put on a third while awaiting intervention.

4.3.3 Bloods

Troponin

As noted in the results chapter, troponin was not detected in any of the blood samples collected. This is not unexpected as the subjects were stable and did not have acute coronary events during the study. Neither did the patients have heart failure or renal impairment which have had associated troponin release, as these were exclusion criteria for the study.

Oxidised LDL

Interestingly the median oxidised LDL was noted to fall 4 hours after the administration of the study drug in both the active and placebo arm and the fall was greatest in the high dose arm. The oxidised LDL levels then rose at 24 hours, rising most in the active arms, which was when the time to ST depression and total exercise time improved most. This again is difficult to rationalise as the time to ST depression fell at four hours after the study drug was administered. As noted in section 1.8, increasing levels of oxidised LDL correlated with severity of CAD so the fact the levels fell at the point where ischaemia is demonstrated more quickly is an unusual finding. Oxidised LDL is however a fairly inexact and insensitive test of tissue oxidative stress and therefore we should not assume too much from these findings.

CRP

There was no clear trend in the CRP across the arms of the study.

BNP

Interestingly in the treatment arms of the study the BNP did show a trend towards a lower reading in the later visits of the active arms compared with the placebo arm. The median BNP rose slightly at 4 hours in all three arms which was when the time to ST depression was

shorter in the active arms, then fell at 24 hours and further on the final visit. This is interesting because as described in the introduction, section 1.10, elevated levels of BNP are associated with inducible ischaemia. However, it would have been expected BNP would fall at 24 hours when the time to ST was longest in the treatment arms and then come back closer to baseline as the time to ST depression shortened again. One explanation is that allopurinol exerted an anti-ischaemic effect by the end of the arm resulting in the BNP levels going down.

4.4 ETT as outcome measure

Exercise treadmill tests have been used for more than six decades to detect CAD.[89, 90] Current guidelines advise categorizing patients as low, intermediate or high risk. High risk patients are then advised to undergo coronary angiography, intermediate risk patients to undergo a stress test such as myocardial perfusion scan or stress echo while low risk patients should not be investigated further.[7] Despite this, ETT remains a widely used approach being used routinely in 59% of chest pain clinics in the UK [91] and was part of the initial assessment of 76% of patients with chest pain in a Euro heart survey. [92] One study found that evaluation of individuals without known CAD, who presented with stable chest pain, using ETT as first line and further tests only if ETT equivocal, was a cost effective approach.[93] An ETT gives additional information on exercise capacity, cardiorespiratory fitness and prognosis. For example, the Duke Treadmill Score, which was initially used as a prognostic score, has been tested as diagnostic score and was found to predict coronary arterial disease more accurately than ST response alone. [94]

Several factors can affect the specificity of ETT, resulting in false positive results, including metabolic conditions, structural heart

conditions and some medications.[95] Furthermore the pattern of underlying CAD affects the specificity and sensitivity of the test. It is reported in patients who subsequently underwent coronary angiography, the sensitivity of ETT was approximately 68% and specificity 77%.[95] This paper also reported the sensitivity for patients with single vessel disease varied from 25% to 71% and was most sensitive in patients with a lesion of left anterior descending artery. In patients with multi-vessel CAD, the sensitivity was reported as 86% and specificity 66%. In addition, ETT is less accurate in women due to an increased number of false positives. [96]

A key question is whether time to ST depression on ETT is really the best endpoint for clinical trials such as these. As the high failure rate in the initial screening visits show, the changes in clinical practice make finding patients with predictable ST depression on ETT incredibly challenging. It may be that using total exercise time as an alternative outcome would allow continued use of ETT findings as an outcome measure.

There are several alternative methods to assess for coronary arterial disease causing cardiac ischaemia which include single-photon emission computed tomography (SPECT), stress echo, cardiac magnetic

resonance imaging, coronary CT angiography and fractional flow reserve derived from coronary CT angiography. A meta-analysis comparing these modalities [97] found cardiac MRI performed well in diagnosing haemodynamically significant coronary arterial disease on both a per-patient as well as per-vessel basis. Cardiac CT and fractional flow reserve CT had a high diagnostic sensitivity, with a low specificity for cardiac CT. The diagnostic performance for the other modalities lists above was generally found to be poorer.

Other studies have shown promising findings for the use of cardiac MRI perfusion scanning to assess for myocardial ischaemia and suggest this modality could be used as a first- line imaging modality for the exclusion of haemodynamic relevant coronary artery stenosis in patients with suspected coronary artery disease (CAD). [98] This modality can also give information on the haemodynamic relevance of coronary stenosis and guide subsequent revascularisation. However MRI is expensive, time consuming and requires the patients to comply. Using this as a repeated test to look for impact on inducible ischaemia would not be practical.

Cardiac CT and SPECT both involve exposure to radiation and therefore again are not appropriate methods of repeat assessment at short

intervals due to cumulative toxic effects of radiation exposure. This is also true for myocardial perfusion scans.

Stress echo is easily accessible but requires the patient to have good echo windows and the person reporting the scan to be highly skilled in order to pick up subtle changes in left ventricular wall motion. It can be seen therefore that all the current modalities to assess for inducible ischaemia have benefits and limitations.

Alternatively should we consider giving up methods of assessing ischaemia in these patients and rely on angina diaries. The low return of completed diaries suggest compliance with this approach can also be a challenge.

Overall ETT remains a reasonable test to assess for ischaemia in chest pain and for the purpose of this study was an easy, accessible method of evaluating the effects of allopurinol on the primary and secondary ETT outcomes. Certainly ETT parameters have been the cornerstone of clinical trials to assess the more recent anti-anginal medications to be marketed. [84, 99-101]

4.5 High intensity exercise training

Several of the study participants reported they found the ETT easier to do as the trial progressed and this is reflected in the significant improvement in mean total exercise time across all the arms of the trial from baseline ETT to final ETT irrespective of whether it was an active or placebo arm. One participant felt so much improved by the end of the trial she actually went out and bought a treadmill for the house while a second joined the local gym so he could continue to exercise. The study involved the participants doing from 3-10 mins of quite intensive exertion three times per week, which was essentially a form of high intensity training (HIT). HIT has been the subject of research in many conditions over recent years including cardiovascular disease.

Cardiac rehabilitation in the form of exercise is a well-established intervention to improved quality of life and survival in coronary artery disease.[102-105] However the most efficient modality and intensity remains unclear. Some studies suggest HIT improves cardiovascular fitness and quality of life[104 106]although a recent meta-analysis concluded there was insufficient evidence at present.[102]

As noted in the introduction (1.1.5) endothelial dysfunction is believed to play a role in the development of atherosclerosis. Therefore interventions that prevent or slow endothelial dysfunction are important in reducing cardiovascular risk. [10] It has been shown exercise training in older adults helps to preserve endothelial function. This adaptive response is explained by the sheer stress induced during elevated blood flow results in vascular adaptation.[10] It has also been shown that in patients with CAD, a reversal of the pathological remodelling and systolic and diastolic improvements were observed only after high-intensity exercise training.[105]

4.6 Challenges

As discussed in section 4.1, recruitment was by far the most challenging aspect of this study. Despite extensive efforts to maximise recruitment the study remained under-recruited. This was largely due to the change in clinical practice as described previously. Interestingly, despite the visit schedule being quite demanding, most patients did complete the study unless they had to withdraw for health reasons. Furthermore a couple of subjects were quite disappointed when the study concluded as they enjoyed interacting with the team.

In addition analysis of the findings of this study were difficult to interpretation due to variability in the sample in terms of current drug therapy, baseline fitness frequency of angina. Furthermore attempting to answer two questions in terms of optimal dose and timing on onset of allopurinol made the data more difficult to interpret.

4.7 Learning points

- Changes in local practice must be considered when considering recruitment for trials

- It may be better to run a pilot study to ascertain ease of recruitment prior to commencing large study
- Attempting to answer more than one question as primary outcome makes analysing data more challenging.
- Reduction of variability in sample would make data analysis easier.

4.8 Summary

This study was underpowered and so it is difficult to draw significant conclusions but it does appear at 24 hours there was an impact on both time to ST depression and total exercise time which warrants further investigation. This was noted despite the fact the uric acid levels did not fall until the end of the treatment arms.

Our protocol was unusual because we were trying to establish a parameter which is not usually considered, namely the time of onset of the anti-ischaemic effect.

It was shown that regular high intensity exercise improved the total exercise time irrespective of whether the treatment arm was active or placebo. This may suggest the protocol was contaminated by the training effect of repeated high intensity exercise. As discussed above, other methods of establishing ischaemia have their limitations; for many, sequential assessment is prohibitive due to exposure to radiation.

It seems to be clear that allopurinol does not exert an anti-ischaemic effect in the first four hours after administration. This means that even if it were to become an established treatment in acute coronary

syndromes in the future it would not need to be delivered very quickly. Furthermore it would be unlikely to be worth formulating allopurinol as a sublingual spray for the management of acute coronary syndromes or even to relieve chest pain during an acute episode of exercise induced angina.

Further work is required to answer the questions asked by this study but trial design would have to be rethought to address the challenges of difficult recruitment and training effects which have been identified by this study.

5 Appendix

A Letter of Invitation

UNIVERSITY OF DUNDEE

Medical Research Institute

DIVISION OF CARDIOVASCULAR AND DIABETES MEDICINE

Head of Division and Professor of Cardiovascular Medicine
Professor Allan D Struthers BSc MD FRCP FESC FRSE FMedSci

«AddressBlock»

«GreetingLine»

Allopurinol as a possible new therapy for acute coronary syndromes:

The Next Steps (The ALL ACS study)

The centre for Cardiovascular and Lung Biology at Ninewells Hospital and Medical School is recruiting patients with chronic angina to a clinical trial called ALL ACS.

We are writing to ask whether you would be willing to find out more about this trial and to consider taking part. The trial will investigate whether a drug called Allopurinol, a safe drug that has been used to treat gout for many years, helps patients with chronic angina. Our theory is that this medication may also help patients with acute angina but before we study acute patients, we need to study chronic angina patients to establish how quickly our drug works (and the best dose to use).

ALL ACS aims to recruit 66 patients. It is being funded by the British Heart Foundation.

We have enclosed an information sheet related to the trial and if you are interested please read it.

Should you be interested in taking part in this trial, please could you either call to discuss further (Telephone no: 01382 632180) or should you prefer, complete the attached reply slip and return in the enclosed envelope.

Many thanks and kind regards

Dr Fiona Shearer
Principal Investigator

B Patient Information Sheet

PARTICIPANT INFORMATION SHEET

Title of Study:

Allopurinol as a possible new therapy for acute coronary syndromes: The Next Steps

(The ALL ACS study)

Name of Researcher:
Chief Investigator: Professor Allan D Struthers
Principal Investigator: Dr Fiona Shearer

Details of Study:
You are being invited to participate in a clinical trial at the Centre for Cardiovascular and Lung Biology, Ninewells Hospital and Medical School. This study will form part of a higher degree for Dr Shearer. Before you decide whether or not to take part it is important for you to understand why the research is being carried out and what it involved. Please take time to read the following information carefully and discuss it with others if you wish. Please ask if there is anything that is unclear or if you would like more information. Take your time to decide whether or not you would like to take part.

Background
Coronary artery disease refers to narrowing in the arteries supplying blood to the heart muscle. It is most commonly experienced by patients as a cramping pain in the chest (angina) that comes that comes on whilst exercising and settles with resting or use of GTN spray. Blood carries oxygen round the body and because of the narrowing in the arteries this means that that the heart muscle does not get as much oxygen as it needs, causing the angina pain. It has recently been found that a medication called allopurinol – a safe drug that has been used to treat gout for many years helps patients with chronic angina. Our theory is that this same medication may also help patients with acute angina but before we study acute patients, we need to study chronic angina patients to establish how quickly our drug works (and the best dose to use).

Do I have to take part?
Participation in this study is entirely voluntary and you are free to refuse to take part or withdraw from the study at any time (without having to give a reason) and without this in any way affecting your future medical care or your relationship with medical staff looking

after you. Some insurance companies consider that participation in medical research such as this is a "material fact" which should be mentioned in any proposal for health-related insurance or which could influence their judgement in consideration of claims under existing policies. You should check that participation in this research does not affect any policy you might be thinking about taking out or any existing policy.

What is involved in the study?

The study takes between nine week and 21 weeks for you to complete.
It is a randomised, double-blind, dose defining, cross over study.

This means that you will be assigned to three different drug regimes for one week at a time with one to four weeks washout or rest period between each change in drug therapy dependent on your availability.
You will be given a tablet which contains the medication we are testing (called allopurinol) or an inactive tablet (called a placebo). We plan to test two different dosing regimes of allopurinol and to compare this to a placebo also.
This means that you will receive all three drug regimes during the study which is what we mean by a cross over study.
Regime A: a 800mg dose of allopurinol (one tablet) in clinic followed by five days of 400mg twice a day
Regime B; a 400mg dose of allopurinol in clinic followed by 5 days of 300mg twice a day.
Regime C;- a placebo tablet in clinic that looks exactly like the allopurinol tablets, followed by five days of placebo.

You will be randomly assigned to regimes A, B or C so that the tablets allocated to you are decided in a random way (a bit like tossing a coin) such that neither you nor the research staff will know which tablet you are taking at any time until after the study is completed. This ensures that the study results cannot be influenced by knowing whether you are receiving the medication or not.

To make sure you are suitable for the study we first ask you to undertake two exercise treadmill tests (ETT). These will be done within two weeks and at least a week apart and are to ensure that your symptoms are stable. During this test we will monitor your heart activity, by attaching you to an ECG monitor (electrocardiogram) for evidence of any angina starting. If your angina symptoms come on within similar times then we will invite you back for the drug study.
If the times on the ETT are more than 15% apart we will do a third ETT a week after the second one. If the third ETT is more than 15% different from the second you will not be randomised into the drug study.

You will then be invited back for the drug dosing study. On this visit you will have another ETT then one hour later will be given a supervised dose of study drug. You will be asked to

remain in the department for four hours and we will do a second ETT that day to assess any changes in exercise tolerance. We'll also take some baseline blood tests (about 40 ml or 8 teaspoons full). We'll randomly allocate you to either the allopurinol treatment or the placebo and then follow you up regularly after starting treatment. Full details of the study visits are listed at the end of this information sheet, but for the whole trial there will be upto 11 hospital visits over 9 – 21 weeks and we may also call you to check how you are doing on the medication.

Taxi travel will be available to you (at our cost) if you wish or we will reimburse any travelling expenses and car parking fees.

With your agreement we would also like to store the blood samples we take for a period of five years so we can use it to test any new blood markers that become available in the near future.

Medication

The medication used in this study is called allopurinol. It was been around for about 50 years now although mainly for the treatment of gout. It has a good safety record and is generally well tolerated. However, like most medicines, allopurinol occasionally causes side effects. The most common side effect is nausea and some abdominal discomfort which affects less than one in ten of patients on allopurinol. This can be minimised by taking the tablets with food.

Allopurinol causes a skin rash in one in a hundred or less of patients. This may be associated with fever, swollen glands, joint pains, unusual blistering or bleeding. Were any of these symptoms to develop, you should stop taking the tablet immediately and contact the study doctor as soon as possible. You may also seek advice from your GP.

Reports of other side effects of allopurinol are very rare (less than 1 in 10,000 people) and it is not always clear if they are truly related to the treatment. The complete range of reported side effects is set out in a Patient Information Leaflet, a copy of which is attached for your information, but include headache, stomach upset, drowsiness, anaemia. This will be further discussed with you before you make a final decision about taking part in this study.

Placebo tablets are inactive tablets that just look the same as the active tablet but do not contain any of the allopurinol medication. We do not expect you to take any side effects at all whilst taking these. Your usual medication should be taken as normal.

Exercise test

This test involves you walking on a treadmill. We are trying to find out how far and for how long you can walk before you experience changes in your ECG rhythm that indicate to the doctor that your heart muscle is not receiving enough oxygen – the hope is that the

treatment will improve this time and distance. The test will be stopped as soon as this is noted or if you experience any angina pains.

Questionnaires

We will use a questionnaire to ask you questions about your angina pain and to chart any angina episodes you have during the time you are enrolled on the study. Our hope is that we will be able to improve your angina pains (with the active drug).

Contraceptive Advice

Anyone who is pregnant cannot take part in this study. If you are a woman of childbearing age we will need to do a pregnancy test before the study. It is also important that you do not become pregnant during the study. Here is some advice on contraception. To avoid getting pregnant, not having sex at all is obviously effective. If you follow this strictly, no contraception is needed. If not, these are effective types of contraception:

- Combined Oral Contraceptive Pill
- EVRA-osetrogen and progestogen: 'Transdermal Patch'
- Progestogen only pill: 'mini pill'
- Depoprovera injection (medroxyprogesterone acetate)
- Implanon Implant (Etonogestrel)
- Mirena Coil (Intra-Uterine System)
- IUD-copper containing intrauterine device
- Female sterilisation

Male vasectomy is also a good form of contraception but only if the procedure has been checked afterwards by your doctor to make sure it has worked.
No contraception method is 100% reliable by itself. Even surgical sterilisation in men and women has been known to fail very occasionally. We advise using additional contraception from the start of the study.

You may normally use 'barrier methods' such as the condom, diaphragm or cap. There is no definite proof that using a spermicide with a 'barrier method' gives extra protection but some condoms are manufactured with spermicide on them. If you require further advice on contraception, please ask.

What are the discomforts, risks and side effects?

The side effects of the allopurinol are discussed under the 'medication' section above.
Having blood tests taken can cause some mild bruising.
You may already routinely use GTN spray for your angina and will be asked to use this if you experience any angina symptoms. This can cause a slight headache although this usually passes quickly.

What are the benefits of taking part in the study?
You will be monitored closely during the study and will be seen by a heart specialist at each of your study visits. Besides having tests that have already been mentioned, your medication will be reviewed on a regular basis. The tests will give us information about the function of your heart, kidneys and blood circulation. If any of these investigations reveal any new abnormality we will either discuss this with your GP or refer you to a specialist clinic at Ninewells Hospital (whichever seems most appropriate). The study may not immediately benefit you, but if the results of the study are positive this may change the practice of managing patients with acute coronary syndrome like you and potentially will have a great impact on other such patients in the future. If so, you may gain eventually from our discovering a new treatment for your condition.

What are my rights?
If you wish the results of the study can be made available to you or your GP when the study is complete.

If you have a complaint about your participation in the study you should first talk to the Investigator involved in your care. You can ask to speak to a senior member of the Centre for Cardiovascular and Lung Biology or the Complaints Officer for NHS Tayside.

Complaints and Claims Manager
Complaints and Advice Team
Level 7, Ninewells Hospital
Dundee DD1 9SY
Freephone: 0800 027 5507
Email: nhstaysidecomplaints@thb.scot.nhs.uk

In the event that something goes wrong and you are harmed during the study there are no special compensation arrangements. If you are harmed and this is due to someone's negligence then you may have grounds for a legal action for compensation against the University of Dundee or NHS Tayside but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you (if appropriate).

Will the research influence the treatment I receive?
The research will not alter the regular treatments you receive.

Will my taking part in the study be kept confidential?
With your permission, identifiable information about you and data collected during the study will be held by the Centre for Cardiovascular & Lung Biology, Ninewells Hospital and Medical School. All data collected in this study will be coded and stored on a computer system protected by a password only available to the researchers. No one outside the research team will have access to any identifiable information and all identifiable information and data will be kept securely. Your data will be archived securely for at least

five years after the end of study as this is a legal requirement for drug studies. With your permission, we will inform your GP of your participation in this study. It is a requirement of the regulators that your records in this study, together with any other relevant medical records, be made available for scrutiny by appropriate staff from NHS Tayside, University of Dundee (or their appointed third party) and the regulatory authorities.

Will I continue to receive the medication used in this study after it finishes?
No. The study is designed to give an indication of possible benefit from the medicine being tested and it may be some time before we can be sure about how useful it actually is.

Who has reviewed this study?
The East of Scotland (EoSRES) Research Ethics Committee 2, which has the authority to scrutinise proposals for medical research on humans, has examined this study and has raised no objections from the point of view of medical research.
It is a requirement that your records in this research, together with any relevant medical records, be made available for scrutiny by monitors from The University of Dundee, NHS Tayside and by the Regulatory Authorities, whose role it is to check that research is properly conducted and the interests of those taking part are adequately protected.

If you are worried at any time about the research or wish to discuss things generally further, please do not hesitate to contact:

Dr Fiona Shearer
BHF Clinical Research Fellow
Centre for Cardiovascular & Lung Biology
Division of Medical Sciences
Mail Box 2, Ninewells Hospital and Medical School
Dundee DD1 9SY
Tel: (01382) 632180
Email: fiona.shearer@dundee.ac.uk

What will happen to me during the study?
The following is the programme of visits involved in this study - we will provide a taxi or travel expenses for all of the visits.

- Visit 1 (week 0) – screening visit 1
    - consent visit– answer any outstanding questions and complete consent form.
    - Baseline ETT
    - Blood tests
    - Record list of current medications
    - Record of medical history & CAD history

- Visit 2 (week 1) – screening visit 2
    - Second baseline ETT – if stable (<15% variance) then can continue in study
    - Record list of current medications

A third ETT visit may be necessary for some participants

- Visit 3 – Randomisation visit (Day 0)
    - ETT one hour before study drug dosing
    - Dosing with Allopurinol/Placebo
    - 2nd ETT after 4 hours of study drug dosing
    - Record list of current medications
    - Supply of full dose study medication x 5 days
    - Issue angina diary to participants

- Visit 4 progress visit (Day 1)
    - ETT
    - Monitor for adverse events
    - Safety Bloods
    - Review angina symptom diary

- Visit 5– progress visit (Day 5)
    - Assess medication compliance.
    - ETT
    - Monitor for adverse events
    - Safety blood check-
    - Review angina symptom diary

      ONE TO FOUR WEEK WASHOUT PERIOD (No drug treatment or hospital visits)

- Visit 6 Randomisation visit (Day 0)
    - ETT one hour before study drug dosing
    - Dosing with Allopurinol/Placebo
    - 2nd ETT within 4 hours of study drug dosing
    - Record list of current medications
    - Supply of full dose study medication x 5 days

- Issue angina diary to participants
- Monitor for adverse events

- Visit 7– progress visit (Day 1)
  - Assess medication compliance.
  - ETT
  - Monitor for adverse events
  - Safety blood check-
  - Review angina symptom diary
- Visit 8– progress visit (Day 5)
  - Assess medication compliance.
  - Monitor for adverse events
  - Safety blood check-
  - Review angina symptom diary

ONE TO FOUR WEEK WASHOUT PERIOD (No drug treatment or hospital visits)

- Visit 9 Randomisation visit (Day 0)
  - ETT one hour before study drug dosing
  - Dosing with Allopurinol/Placebo
  - 2nd ETT within 4 hours of study drug dosing
  - Record list of current medications
  - Supply of full dose study medication x 5 days
  - Issue angina diary to participants
  - Monitor for adverse events
- Visit 10– progress visit (Day 1)
  - Assess medication compliance.
  - ETT
  - Monitor for adverse events
  - Safety blood check-
  - Review angina symptom diary

- Visit 11– final visit (Day 5)
    - ETT
    - Assess medication compliance.
    - Blood tests
    - Monitor for adverse events
    - Record list of current medications

Contact Numbers

If during the study you become unwell or are concerned, as well as the usual services provided by the NHS such as NHS24 (08454 242424), you can also contact the study team during normal working hours on (01382) 632180. If you are unwell and need urgent advice or assistance do not delay in seeking further advice or treatment as usual through the NHS services.

Thank you for reading this information sheet and considering taking part in this study. If you would like more information or want to ask questions about the study please contact the study team on the number above.

ALL ACS PARTICIPANT STUDY SCHEDULE

	Screening				Randomisation A			Washout period	Randomisation B				Randomisation C		
CLINIC VISIT	Visit 1	Visit 2	Visit 2a * Repeat ETT	Pre randomisation Period	Visit 3	Visit 4 Day 1 F/up	Visit 5 Day 5 f/up		Visit 6	Visit 7 Day 1 F/up	Visit 8 Day 5 f/up	Washout Period	Visit 9	Visit 10 Day 1 f/up	Visit 11 Final Visit
				1-4 weeks	Day 0	Day 1	Day 5	1-4 weeks	Day 0	Day 1	Day 5	1-4 weeks	Day 0	Day 1	Day 5
Informed Consent	X														
Medical & Family History	X														
Phyiscal Examination	X														
Blood pressure check	X				X	X	X		X	X	X		X	X	X
Exercise Tolerance Test	X	X	X*		X[a] X[b]	X	X		X[a] X[b]	X	X		X[a] X[b]	X	X
Study Drug dosing					X				X				X		
Blood Test	X				X	X	X		X	X	X		X	X	X
Receive 5 days Study medication					X				X				X		
Receive/ Review Angina Diary					X	X	X		X	X	X		X	X	X
Adverse Event Assessment					X	X	X		X	X	X		X		
Review Medications with doctor	X				X	X	X		X	X	X		X	X	X

Vist 2a*- required only if screening and visit 2 ETT are not compatible.
X[a] – 1 hour pre study drug
X[b]- 4 hours post study drug

C Patient Reply Slip

Please complete the details below and return in the enclosed envelope or if you would prefer, please contact the study Doctor on the following number:

Dr Fiona Shearer Tel no: 01382 383013

I am interested in taking part in the ALL ACS trial and I agree to be contacted by a member of the research team. ☐

Name:

Address:

Date of Birth:

Tel no:

Date:

Many thanks for your time

D Patient Appointment Letter

UNIVERSITY OF DUNDEE

Medical Research Institute

DIVISION OF CARDIOVASCULAR AND DIABETES MEDICINE

Head of Division and Professor of Cardiovascular Medicine
Professor Allan D Struthers BSc MD FRCP FESC FRSE FMedSci

«AddressBlock»

Allopurinol as a possible new therapy for acute coronary syndromes:

The Next Steps (The ALL ACS study)

«GreetingLine»

I am writing to confirm your appointment on «screening_date_».

To get to the department remain on level 7 (the level on which you enter the hospital), turn left at the café and head towards wards 1-6. Continue past the entrance to wards 5&6 and you will come to double doors on your left. Come through these doors and ring the doorbell beside the next set of double doors which will be in front of you.

I look forward to seeing you.

Many thanks and kind regards

Dr Fiona Shearer
Principal Investigator

NINEWELLS HOSPITAL AND MEDICAL SCHOOL · Mail Box 2 · Dundee DD1 9SY Scotland UK
t +44 (0)1382 383013 *f* +44 (0)1382 644972 *e* a.d.struthers@dundee.ac.uk

E Patient Reply Slip

UNIVERSITY OF DUNDEE

Medical Research Institute

DIVISION OF CARDIOVASCULAR AND DIABETES MEDICINE

Head of Division and Professor of Cardiovascular Medicine
Professor Allan D Struthers BSc MD FRCP FESC FRSE FMedSci

Study Number: 2011-004996-35 (EudraCT)

Participant Identification Number for this trial: .....

PARTICIPANT CONSENT FORM

Title of Study: Allopurinol as a possible new therapy for acute coronary syndromes: The Next Steps

(The ALL ACS study)

Name of Researcher:
Chief Investigator: Professor Allan D Struthers
Principal Investigator: Dr Fiona Shearer

Please initial box

1. I confirm that I have read and understand the information sheet dated _____ (version ___) for the above study. I have had the opportunity to consider the information, to ask questions, and have had them answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by the research team or from the regulatory authorities, NHS Tayside, or the University of Dundee (or their appointed third party), where it is relevant to my taking part in this study. I give permission for these individuals to have access to my records. ☐

4. I agree to my GP being informed of my participation in this study. ☐

5. I agree to take part in the above study. ☐

Name of participant	Date	Signature
Name of person taking consent	Date	Signature

1 copy for participant; 1 copy for trial master file; 1 original to be kept with hospital notes

NINEWELLS HOSPITAL AND MEDICAL SCHOOL · Mail Box 2 · Dundee DD1 9SY Scotland UK
t +44 (0)1382 383013 f +44 (0)1382 644972 e a.d.struthers@dundee.ac.uk

F GP Letter

UNIVERSITY OF DUNDEE

Medical Research Institute

DIVISION OF CARDIOVASCULAR AND DIABETES MEDICINE

Head of Division and Professor of Cardiovascular Medicine
Professor Allan D Struthers BSc MD FRCP FESC FRSE FMedSci

30/07/2014

Dear

Title of Project: Allopurinol as a possible new therapy for acute coronary syndromes: The Next Steps

ALL ACS- (ALLOPURINOL IN ACUTE CORONARY SYNDROME)

Patient:

The patient named above has agreed to take part in the above clinical research study.

This randomised placebo controlled clinical trial of allopurinol in Acute Coronary Syndrome has three arms as described in the attached Patient Information Sheet.

Your patient will receive one of two different doses of allopurinol or placebo for one week at a time with a one week wash out period between.

Allopurinol has anti-ischaemic/oxygen sparing effects in chronic stable angina (CSA) by protecting the heart during oxygen deficiency. This means that it may also be very beneficial in acute coronary syndromes (ACS) by reducing cardiomyocyte necrosis. However, in order for a definitive megatrial of allopurinol in ACS to be designed optimally, we need to answer two key questions. Firstly, how quickly does allopurinol begin to exert its oxygen sparing effect and secondly what is the optimum dose? To answer these questions, we will perform exercise tests in CSA patients after varying doses of allopurinol and repeat them at certain time intervals in order to see how quickly an anti-ischaemic effect of allopurinol comes on.

Your patients participation is this study will last for up to 9 weeks.

I have enclosed a patient information sheet which gives full study details, however if there are any questions you may have regarding the study, I would be happy for you to contact me.

Yours faithfully

Dr Fiona Shearer
Clinical Research Fellow

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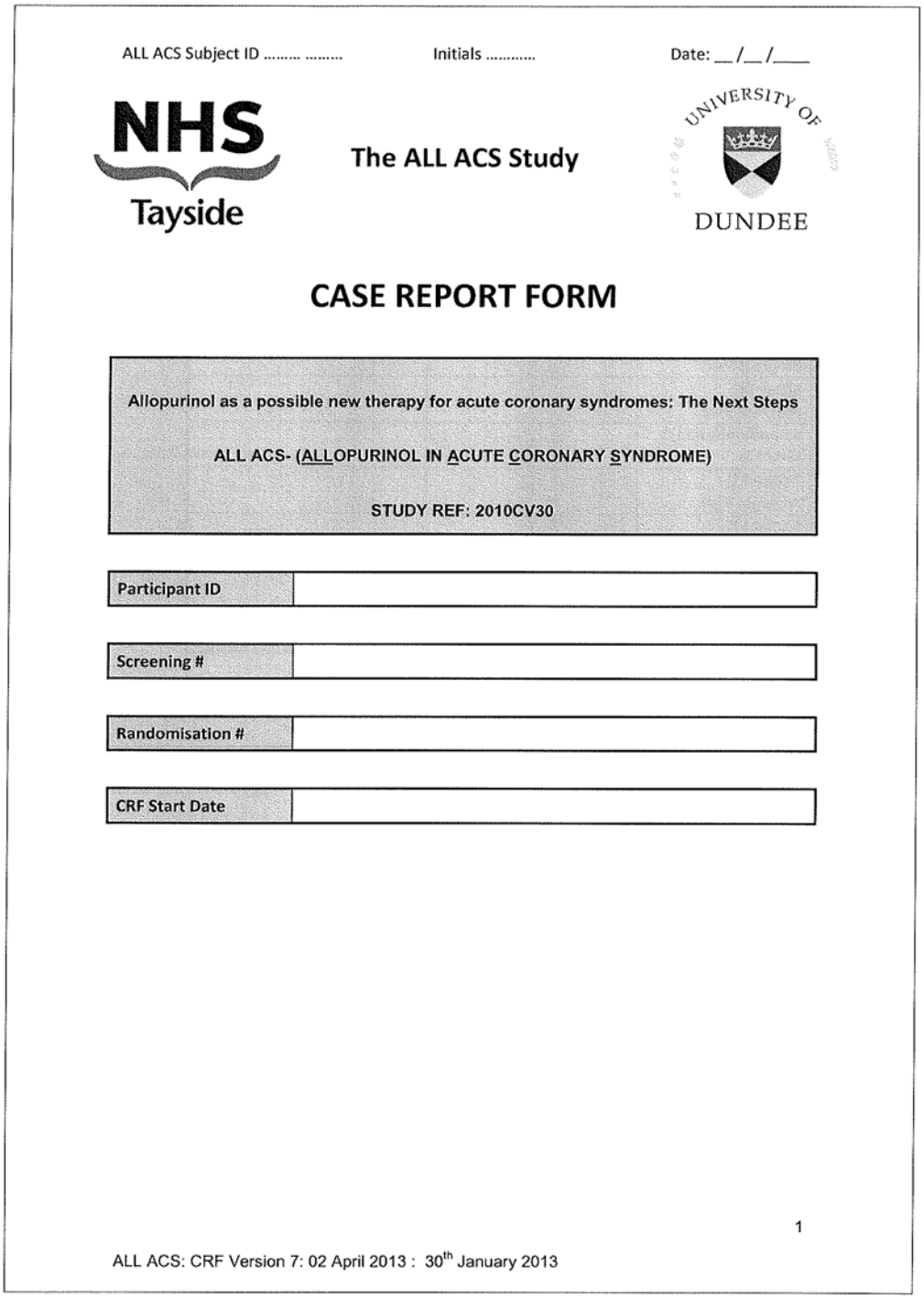

ALL ACS Subject ID .... Initials ..... Date: __/__/____

NHS Tayside

The ALL ACS Study

UNIVERSITY OF DUNDEE

CASE REPORT FORM

Allopurinol as a possible new therapy for acute coronary syndromes: The Next Steps

ALL ACS- (ALLOPURINOL IN ACUTE CORONARY SYNDROME)

STUDY REF: 2010CV30

Participant ID	
Screening #	
Randomisation #	
CRF Start Date	

1

ALL ACS: CRF Version 7: 02 April 2013 : 30th January 2013

G Case Report Form

ALL ACS Subject ID .... Initials ..... Date: __/__/____

Visit	Date	Taxi Required?	Taxi Booked?	Comment
1 Screening 1				
2 Screening 2				
2a Screening				
3 Randomisation A (Day 0)				
4 (Day 1) Progress Visit				
5(Day 5) Progress Visit				
6 Randomisation B (Day 12)				
7 (Day 13) Progress Visit				
8 (Day 17) Progress Visit				
9 Randomisation C (Day 24)				
10 (Day 25) Progress Visit				
11 (Day 29) Final Visit				

ALL ACS Subject ID .... Initials ..... Date: __ /__ /____

Study visits overview

- Visit 1– screening visit 1 (Day -14 to -21)
  - Participant consent – answer any outstanding questions and complete consent form.
  - Check Inc/Exc Criteria
  - Medical History
  - Family History
  - Clinical Examination
  - Baseline ETT
  - Bloods for FBC/UE/LFTs
  - Record list of current medications

- Visit 2 (Day -7 to -14) – screening visit 2
  - Second baseline ETT – if stable (Onset of ST depression of greater than 1mm is <15% compared with ETT 1) then can continue in study **Go to Visit 3**

- Visit 2a (Day -7 to -1) – screening visit 3
  - Third baseline ETT (Only required if ST depression between ETT1 & ETT is > 15% variance) (If Onset of ST depression of greater than 1mm is <15% compared with ETT 2) then can continue in study
  - **SCREEN FAIL** If >15% variance

- Visit 3 (Day 0) – Randomisation A visit
  - Bloods for FBC/UE/LFT/uric acid/BNP/hsCRP/Troponin /isoprostanes/oxidised LDL pre 1st ETT
  - Baseline ETT 1 hour pre study drug stat dose
  - Administer stat dose study drug
  - Blood test : Uric acid: (2 hour post study drug)
  - Bloods for uric acid/BNP/hsCR/Troponin /isoprostanes/oxidised LDL: pre 2nd ETT (4 hours post study drug)
  - Blood test for uric acid/BNP/Troponin: post 2nd ETT
  - Check and note AEs
  - Record list of current medications
  - Issue Angina diary
  - Supply of 5 days study medication
  -

- Visit 4 (day 1) – progress visit
  - Bloods for FBC/UE/LFT/BNP/hsCRP/Troponin /isoprostanes/oxidised LDL: pre ETT
  - ETT
  - Bloods for BNP/hsCR/Troponin /isoprostanes/oxidised LDL: post ETT
  - Assess medication compliance.
  - Check for AEs
  - Check Angina diary
  - Record any changes to current medications

- Visit 5 (day 5) – progress visit
  - Bloods for FBC/UE/LFT/BNP/uric acid/hsCRP/Troponin /isoprostanes/oxidised LDL pre ETT
  - ETT
  - Bloods for uric acid/ Troponin: post ETT
  - Assess medication compliance and get unused study drug back
  - Check for AEs
  - Check Angina diary
  - Record any changes to current medications

- Visit 6 (Day 12) – Randomisation B visit
  - Bloods for FBC/UE/LFT/uric acid/BNP/hsCRP/Troponin /isoprostanes/oxidised LDL: pre 1st ETT
  - Baseline ETT 1 hour pre study drug stat dose
  - Administer stat dose study drug
  - Blood test : Uric acid: (2 hour post study drug)
  - Bloods for uric acid/BNP/hsCR/Troponin /isoprostanes/oxidised LDL: pre 2nd ETT (4 hours post study drug)
  - Blood test for uric acid/BNP/Troponin: post 2nd ETT
  - Check and note AEs
  - Record list of current medications

- Issue Angina diary
- Supply of 5 days study medication
-

- Visit 7 (day 13) – progress visit
  - Bloods for FBC/UE/LFT/ BNP/hsCRP/Troponin /isoprostanes/oxidised LDL pre ETT
  - ETT
  - Bloods for BNP/hsCR/Troponin /isoprostanes/oxidised LDL: post ETT
  - Assess medication compliance.
  - Check for AEs
  - Check Angina diary
  - Record any changes to current medications

- Visit 8 (day 17) – progress visit
  - Bloods for FBC/UE/LFT/uric acid/BNP/hsCRP/Troponin /isoprostanes/oxidised LDL:pre ETT
  - ETT
  - Bloods for uric acid/ Troponin: post ETT
  - Assess medication compliance and get unused study drug back
  - Check for AEs
  - Check Angina diary
  - Record any changes to current medications

- Visit 9 (Day 24) – Randomisation C visit
  - Bloods for FBC/UE/LFT/uric acid/BNP/hsCRP/Troponin /isoprostanes/oxidised LDL pre 1st ETT
  - Baseline ETT 1 hour pre study drug stat dose
  - Administer stat dose study drug
  - Blood test : Uric acid: (2 hour post study drug)
  - Bloods for uric acid/BNP/hsCR/Troponin /isoprostanes/oxidised LDL: pre 2nd ETT (4 hours post study drug)
  - Blood test for uric acid/BNP/Troponin : post 2nd ETT
  - Check and note AEs
  - Record list of current medications
  - Issue Angina diary
  - Supply of 5 days study medication

- Visit 10 (day 25) – progress visit
  - Bloods for FBC/UE/LFT/ BNP/hsCRP/Troponin/isoprostanes/oxidised LDL :pre ETT
  - ETT
  - Bloods for BNP/hsCR/Troponin /isoprostanes/oxidised LDL: post ETT
  - Assess medication compliance.
  - Check for AEs
  - Check Angina diary
  - Record any changes to current medications

- Visit 11(day 29) – final visit
  - Bloods for FBC/UE/LFT/uric acid/BNP/hsCRP/Troponin /isoprostanes/oxidised LDL :pre ETT
  - ETT
  - Bloods for uric acid/Troponin: post ETT
  - Assess medication compliance and get unused study drug back
  - Check for AEs
  - Check and retain Angina diary
  - Record any changes to current medications

ALL ACS Subject ID .... Initials ..... Date: __ /__ /____

Visit 1

Visit 1 (Screening visit 1 of 2)

Participant consent – answer any outstanding questions and complete consent form.

V1A	Has a consent form been completed & filed in the TMF?	

Check inclusion/exclusion criteria.

Inclusion

1. Is the subject aged between 18-85 years?	Yes / No
2. Does the subject suffer from acute coronary syndrome, which is defined as:	
• 2.1 Angiographically documented coronary artery disease	Yes / No
• 2.2 A positive exercise tolerance test (ETT)	Yes / No
• 2.3 A history of symptoms of chronic, stable, effort-induced angina for ≥ 2 months	Yes / No

V1B	Does the subject meet inclusion criteria?	

Exclusion

1.Is the subject unable to do an ETT due to back or leg problems?	Yes / No
2. Does the subject have Left Ventricular Ejection Fraction <45% or significant valvular pathology?	Yes / No
3. Does the subject have any atrial arrhythmias or ECG abnormalities interfering with ST-segment interpretation or previous ventricular arrhythmias on ETT?	Yes / No
4. Is the subject known to suffer from malignancy, chronic kidney (estimated GFR <60 ml/min or creatinine >180 mmol/ml) or liver disease?	Yes / No
5. Has the subject had any coronary revascularization (percutaneous or CABG) in the past 6 months?	Yes / No
6. Has the subject had any cardiovascular disease event within the last two months like MI, unstable angina, stroke?	Yes / No
7. Does the subject have other serious illness or significant abnormalities that may compromise their safety or successful participation in the study?	Yes / No
8. Is the subject already on allopurinol or known to have an adverse reaction to it?	Yes / No
9. Is the subject receiving treatment with either 6-mercaptopurine, azathioprine, warfarin, or theophylline?	Yes / No
10. Is the subject pregnant, breast-feeding or a woman of child-bearing potential not using adequate contraception?	Yes / No
11. Does the subject have any illness which in the doctor's opinion means that the subject is unable to give informed consent?	Yes / No
12. Has the subject participated in another clinical trial (other than observational trials and registries) concurrently or within 30 days prior to screening for entry into this study?	Yes / No

V1C	Is the subject free of any exclusion criteria at this stage?	

Unless the answers to questions V1A are all YES and V1B are all NO then the subject may not continue any further in the study.

ALL ACS Subject ID .... Initials ..... Date: __ /__ /____

Visit 1

Visit 1 (continued)

History

Past Medical History – circle yes (Y) or no (N)

			Date Diagnosed
Angina	*Y*	*N*	
MI	*Y*	*N*	
Stroke/TIA	*Y*	*N*	
If yes, select one: haemorrhagic / infarct / unknown			
Treated Hypertension	*Y*	*N*	
Treated Hypercholesterolaemia	*Y*	*N*	

Previous CV interventions: (List with date of diagnosis)

Coronary Artery Disease

LMS

LAD

LCx

RCA

Other relevant Medical History (List with date of diagnosis)

Family History

1st degree relative with MI/CVA before age 65:- yes/no

Relationship : father/mother/brother/sister/son/daughter

Social History

Smoking status:

❑ current smoker ❑ non-smoker ❑ previous smoker Year stopped _____

cigarettes/day (A) _____# years smoked (B) _____

Average weekly alcohol intake: Nil/ Light/ Moderate/ Heavy

Occupation (circle one):

Employed/ Unemployed/ Full or Part time Education/ Retired/ House Person/ Carer/ Not employed due to disability or illness

If employed or unemployed, most recent/current job: Manual/ Non Manual

Home circumstances: Living alone/ Living with other people

ALL ACS Subject ID .... Initials ..... Date: __/__/____

Visit 1

Examination

General	Age _____	Height _____ m	BP ____/____ mmHg
	Gender Male / Female	Weight _____ kg	Pulse _____ bpm
Cardiovascular *(circle)*		***Respiratory*** *(circle)*	
Normal/abnormal		*Normal/abnormal*	
_State abnormality		*_State abnormality*	
Gastrointestinal*(circle)*		***Neurological****(circle)*	
Normal/abnormal		*Normal/abnormal*	
_State abnormality		*_State abnormality*	

V1D	**Medical history (incl. medication list) and physical examination complete**	

Venepuncture

Samples required:

- 1x EDTA (purple 4ml) – FBC
- 1x clotted (yellow 5ml) – UE/LFT

V1E	**Blood samples taken for safety bloods**	

V1F	**Record concomitant medications at back of CRF**	

ALL ACS Subject ID .... Initials ..... Date: __ /__ /____

Visit 1

Visit 1 (continued)

Baseline ETT (ETT 1)

Resting ECG

Normal / Abnormal (circle one)

State any abnormalities: _____

ETT Targets

Target exercise time for age: ___min : ___sec

Target HR for age: ____ bpm

Results

Max HR achieved: ____ bpm

% of target HR achieved: _____ %

Max workload in METS: _____

Stage at ST depression: (1-5) ____

Total exercise time _____

ECG Interpretation

ECG changes (circle one): yes / no

ST depression (circle one) yes / no amount: _____

ST depression >1 mm onset @ ___min : ___sec

Leads with ST changes: _____

Time of onset of other ECG changes: ___min : ___sec

ST elevation (circle one) yes / no amount: _____

Arrhythmias (circle one): yes / no

Type (circle one or more):

CHB / Asystole / VT / VF / Change in morphology

Other _____

Chest Pain

Chest pain (circle one): yes / no

Time to chest pain (if present): ___min : ___sec

ALL ACS Subject ID .... Initials ..... Date: __ /__ /____

Reasons for stopping the treadmill:

- ❑ ST depression >1 mm
- ❑ Other ECG changes, specify reason_____
- ❑ Chest Pain
- ❑ Shortness of breath
- ❑ Leg pain/leg tiredness
- ❑ Hypotension
- ❑ Hypertension
- ❑ Other, specify _____

BP Record

Start: / mmHg	9min: / mmHg
3min: / mmHg	12min: / mmHg
6min: / mmHg	End: / mmHg

V1F ETT carried out and results printed & filed in CRF	

Visit 1 Final checks

V1G Date for next booked and recorded on front of CRF	

Signed		**Name**		**Date**	

ALL ACS Subject ID .... Initials ..... Date: __ /__ /____

Visit 2 (Screening visit 2).

ETT 2

V2A Visit 1 ETT results documented	

Resting ECG

Normal / Abnormal (circle one)

State any abnormalities: _____

ETT Targets

Target exercise time for age: ___min : ___sec

Target HR for age: ____ bpm

Results

Max HR achieved: ____ bpm

% of target HR achieved: _____ %

Max workload in METS: _____

Stage at ST depression: (1-5) ____

Total exercise time _____

ECG Interpretation

ECG changes (circle one): yes / no

ST depression (circle one) yes / no amount: _____

ST depression >1 mm onset @ ___min : ___sec

Leads with ST changes: _____

Time of onset of other ECG changes: ___min : ___sec

ST elevation (circle one) yes / no amount: _____

Arrhythmias (circle one): yes / no

Type (circle one or more):

CHB / Asystole / VT / VF / Change in morphology

Other _____

Chest Pain

Chest pain (circle one): yes / no

Time to chest pain (if present): ___min : ___sec

ALL ACS Subject ID .... Initials ..... Date: __ /__ /____

Visit 2

Reasons for stopping the treadmill:

- ❑ ST depression >1 mm
- ❑ Other ECG changes, specify reason_____
- ❑ Chest Pain
- ❑ Shortness of breath
- ❑ Leg pain/leg tiredness
- ❑ Hypotension
- ❑ Hypertension
- ❑ Other, specify _____

BP Record

Start: / mmHg	9min: / mmHg
3min: / mmHg	12min: / mmHg
6min: / mmHg	End: / mmHg

V2B Calculate ST depression comparison (difference in seconds between ETT1 & ETT2 divided by higher of ETT 1 or 2 multiplied by 100) of greater than 1mm <15% compared to ETT 1	%=

If ETT 1 and 2 are within 15% of each other- proceed to Visit 3- Randomisation visit

If ETT1 and ETT2 are greater than 15% of each other--- reschedule further ETT within 7 days of ETT 2

V2C ETT2 carried out and results printed & filed in CRF	

Signed		**Name**		**Date**	

ALL ACS Subject ID .... Initials ..... Date: __ /__ /____

Visit 2a

Visit 2a (Screening visit 2a).

ETT 2a

V2A	Visit 1 and 2 ETT results compared and documented	

Resting ECG

Normal / Abnormal (circle one)

State any abnormalities: _____

ETT Targets

Target exercise time for age: ___min : ___sec

Target HR for age: ____ bpm

Results

Max HR achieved: ____ bpm

% of target HR achieved: _____ %

Max workload in METS: _____

Stage at ST depression: (1-5) ____

Total exercise time _____

ECG Interpretation

ECG changes (circle one): yes / no

ST depression (circle one) yes / no amount: _____

ST depression >1 mm onset @ ___min : ___sec

Leads with ST changes: _____

Time of onset of other ECG changes: ___min : ___sec

ST elevation (circle one) yes / no amount: _____

Arrhythmias (circle one): yes / no

Type (circle one or more):

CHB / Asystole / VT / VF / Change in morphology

Other _____

Chest Pain

Chest pain (circle one): yes / no

Time to chest pain (if present): ___min : ___sec

Visit 2a

ALL ACS Subject ID .... Initials ..... Date: __ /__ /____

Reasons for stopping the treadmill:

- ❑ ST depression >1 mm
- ❑ Other ECG changes, specify reason_____
- ❑ Chest Pain
- ❑ Shortness of breath
- ❑ Leg pain/leg tiredness
- ❑ Hypotension
- ❑ Hypertension
- ❑ Other, specify _____

BP Record

Start: / mmHg	9min: / mmHg
3min: / mmHg	12min: / mmHg
6min: / mmHg	End: / mmHg

V2B Calculate ST depression comparison (difference in seconds between ETT2 & ETT2a divided by higher of ETT2 or 2a multiplied by 100) of greater than 1mm <15% compared to ETT 2	%=

If ETT 2 and 2a are within 15% of each other- proceed to Visit 3- Randomisation visit

If ETT2 and ETT2a are greater than 15% of each other--- SCREEN FAIL

V2C ETT2a carried out and results printed & filed in CRF	

V2D Less than 15% variance with first baseline ETT confirmed (if NOT then explain to participant why they are unable to progress further in study)	

Signed		**Name**		**Date**	

Visit 3

ALL ACS Subject ID .... Initials ..... Date: __/__/____

Visit 3 Randomisation Visit

V3A	Medications log updated	

Venepuncture PRE 1st ETT
Samples required:

- 1x EDTA (purple 4ml) – FBC
- 1x clotted (yellow 5ml) – UE/LFT's
- 2x gold & 1 x EDTA (purple) – Research Bloods

V3B	Blood samples taken for FBC/UE&LFT/BNP/Storage	

ETT 1-hour pre study drug stat dose

Resting ECG

Normal / Abnormal (circle one)

State any abnormalities: _____

ETT Targets

Target exercise time for age: ___min : ___sec

Target HR for age: ____ bpm

Results

Max HR achieved: ____ bpm

% of target HR achieved: _____ %

Max workload in METS: _____

Stage at ST depression: (1-5) ____

Total exercise time _____

ECG Interpretation

ECG changes (circle one): yes / no

ST depression (circle one) yes / no amount: _____

ST depression >1 mm onset @ ___min : ___sec

Leads with ST changes: _____

Time of onset of other ECG changes: ___min : ___sec

ST elevation (circle one) yes / no amount: _____

Arrhythmias (circle one): yes / no

ALL ACS Subject ID .... Initials ..... Date: __ /__ /____

Visit 3

Type (circle one or more): CHB / Asystole / VT / VF / Change in morphology Other _____
Chest Pain Chest pain (circle one): yes / no Time to chest pain (if present): ___min : ___sec

Reasons for stopping the treadmill:

- ❑ ST depression >1 mm
- ❑ Other ECG changes, specify reason_____
- ❑ Chest Pain
- ❑ Shortness of breath
- ❑ Leg pain/leg tiredness
- ❑ Hypotension
- ❑ Hypertension
- ❑ Other, specify _____

BP Record

Start: / mmHg	9min: / mmHg
3min: / mmHg	12min: / mmHg
6min: / mmHg	End: / mmHg

V3c Baseline ETT 1 hour pre study drug dosing completed	

Medication Issued (Stat Dose)

Study medication supply

Randomisation Number _____

Date and time of stat dose administered _____

Comments _____

_____

_____

V3D Stat dose Medication checked	

Venepuncture 2 hour post study drug

Samples required:

- 1 x clotted (yellow 5ml) uric acid storage

ALL ACS Subject ID .... Initials ..... Date: __ /__ /____

Visit 3

V3E	Blood samples 2 hour post study drug for Uric acid- (storage)	

Venepuncture 4 hour post study drug (PRE 2nd ETT)

Samples required:

- 1 x clotted (yellow 5ml) uric acid, Hs CRP, Troponin
- 2 x EDTA (purple) BNP, oxidised LDL, Isoprostane

V3F	Blood samples 4 hour post study drug (pre 2nd ETT) – Research Bloods	

Visit 3

ETT 2: 4 hours post study drug stat dose

Resting ECG

Normal / Abnormal (circle one)

State any abnormalities: _____

ETT Targets

Target exercise time for age: ___min : ___sec

Target HR for age: ____ bpm

Results

Max HR achieved: ____ bpm

% of target HR achieved: _____ %

Max workload in METS: _____

Stage at ST depression: (1-5) ____

Total exercise time _____

ECG Interpretation

ECG changes (circle one): yes / no

ST depression (circle one) yes / no amount: _____

ST depression >1 mm onset @ ___min : ___sec

Leads with ST changes: _____

Time of onset of other ECG changes: ___min : ___sec

ST elevation (circle one) yes / no amount: _____

Arrhythmias (circle one): yes / no

ALL ACS Subject ID .... Initials ..... Date: __ /__ /____

Type (circle one or more): CHB / Asystole / VT / VF / Change in morphology Other _____
Chest Pain Chest pain (circle one): yes / no Time to chest pain (if present): ___min : ___sec

Visit 3

Reasons for stopping the treadmill:

- ❑ ST depression >1 mm
- ❑ Other ECG changes, specify reason_____
- ❑ Chest Pain
- ❑ Shortness of breath
- ❑ Leg pain/leg tiredness
- ❑ Hypotension
- ❑ Hypertension
- ❑ Other, specify _____

BP Record

Start: / mmHg	9min: / mmHg
3min: / mmHg	12min: / mmHg
6min: / mmHg	End: / mmHg

V3G ETT 4 hour post study drug dosing completed	

- 1 x EDTA (purple) BNP
- 1 x clotted (yellow 5ml) uric acid, Troponin

V3H Post 2nd ETT bloods – uric acid, BNP, Troponin	

V3I Adverse Events recorded	

Study medication supply

Number of tablets supplied _____

Randomisation Number _____

V3J Bottle of study medication issued, complete with instructions	

ALL ACS Subject ID .... Initials ..... Date: __ /__ /____

V3K	Angina Diary issued, complete with instructions	

Final Visit 3 checks

V3L	Date for fourth visit booked and recorded on front of CRF	

Signed		Name		Date	

Visit 4

ALL ACS Subject ID .... Initials ..... Date: __/__/____

Visit 4 (Day 1 – Progress Visit) **Visit 3 safety bloods results:**

Venepuncture PRE ETT

Samples required:

- 3 x EDTA (purple 4ml) – FBC, BNP, oxidised LDL, Isoprostane
- 2x clotted (yellow 5ml) – UE/LFT, hs CRP, Troponin

V4B Blood samples taken for FBC/U&Es/LFT/BNP/Troponin/hsCRP/isoprostane/oxidised LDL

Resting ECG

Normal / Abnormal (circle one)

State any abnormalities: _____

ETT Targets

Target exercise time for age: ___min : ___sec

Target HR for age: ____ bpm

Results

Max HR achieved: ____ bpm

% of target HR achieved: _____ %

Max workload in METS: _____

Stage at ST depression: (1-5) ____

Total exercise time _____

ECG Interpretation

ECG changes (circle one): yes / no

ST depression (circle one) yes / no amount: _____

ST depression >1 mm onset @ ___min : ___sec

Leads with ST changes: _____

Time of onset of other ECG changes: ___min : ___sec

ST elevation (circle one) yes / no amount: _____

Arrhythmias (circle one): yes / no

Type (circle one or more):

CHB / Asystole / VT / VF / Change in morphology

Other _____

Chest Pain

Chest pain (circle one): yes / no

Visit 4

ALL ACS Subject ID .... Initials ..... Date: __ /__ /____

Time to chest pain (if present): ___min : ___sec

Reasons for stopping the treadmill:

- ❑ ST depression >1 mm
- ❑ Other ECG changes, specify reason_____
- ❑ Chest Pain
- ❑ Shortness of breath
- ❑ Leg pain/leg tiredness
- ❑ Hypotension
- ❑ Hypertension
- ❑ Other, specify _____

BP Record

Start: / mmHg	9min: / mmHg
3min: / mmHg	12min: / mmHg
6min: / mmHg	End: / mmHg

Venepuncture 2 hour post study drug

Samples required:

- 2 x EDTA (purple 4ml) BNP, oxidised LDL, Isoprostane
- 1x clotted (yellow 5ml) hs CRP, Troponin

V4C	**Blood samples post ETT for BNP/hsCRP/Troponin/isoprostane/oxidised LDL**	

Medication compliance

Number of tablets remaining _____

Comments _____

_____

V4D	**Medication compliance checked**	

V4E	**Adverse event log updated**	

V4F	**Angina Diary; checked**	

V4G	**Medications log updated**	

Final Visit 4 checks

V4H	**Date for next visit booked and recorded on front of CRF**				
Signed		**Name**		**Date**	

ALL ACS Subject ID .... Initials ..... Date: __ /__ /____

Visit 5

Visit 5 (Day 5 – Progress Visit)

Visit 4 safety bloods results: **CS/NCS (circle)**

V5A Visit 4 blood results documented : any that are CS- note on AE form	

Venepuncture PRE ETT

Samples required:

- 3x EDTA (purple 4ml) – FBC, BNP **isoprostane/oxidised LDL**
- 2x clotted (yellow 5ml) – UE/LFT/BNP/storage

V5B Blood samples taken for FBC/U&Es/LFT/BNP/ uric acid/Troponin/hsCRP/isoprostane/oxidised LDL	

Resting ECG

Normal / Abnormal (circle one)

State any abnormalities: _____

ETT Targets

Target exercise time for age: ___min : ___sec

Target HR for age: ____ bpm

Results

Max HR achieved: ____ bpm

% of target HR achieved: _____ %

Max workload in METS: _____

Stage at ST depression: (1-5) ____

Total exercise time _____

ECG Interpretation

ECG changes (circle one): yes / no

ST depression (circle one) yes / no amount: _____

ST depression >1 mm onset @ ___min : ___sec

Leads with ST changes: _____

Time of onset of other ECG changes: ___min : ___sec

ST elevation (circle one) yes / no amount: _____

Arrhythmias (circle one): yes / no

Type (circle one or more):

CHB / Asystole / VT / VF / Change in morphology

Other _____

Visit 5

ALL ACS Subject ID .... Initials ..... Date: __/__/____

Chest Pain

Chest pain (circle one): yes / no

Time to chest pain (if present): ___min : ___sec

Reasons for stopping the treadmill:

- ❑ ST depression >1 mm
- ❑ Other ECG changes, specify reason_____
- ❑ Chest Pain
- ❑ Shortness of breath
- ❑ Leg pain/leg tiredness
- ❑ Hypotension
- ❑ Hypertension
- ❑ Other, specify _____

BP Record

Start: / mmHg	9min: / mmHg
3min: / mmHg	12min: / mmHg
6min: / mmHg	End: / mmHg

Venepuncture 2 hour post study drug

Samples required:

- 1 x clotted (yellow 5ml) uric acid/BNP/storage

V5C	**Blood samples post ETT for uric acid/troponin**

Medication compliance

Number of tablets remaining _____

Comments _____

_____

_____

V5D	**Medication compliance checked & Unused medications returned to store**
V5E	**Adverse event log updated**
V5F	**Angina Diary; checked**
V5G	**Medications log updated**

Final Visit 5 checks

ALL ACS Subject ID .... Initials ..... Date: __ /__ /____

V5H	Date for next visit booked and recorded on front of CRF	

Signed		Name		Date	

ALL ACS Subject ID .... Initials ..... Date: __/__/____

Visit 6

Visit 6 Randomisation Visit (Day 12)

	Visit 5 safety bloods results:	CS/NCS (circle)
V6A	Medications log updated: any that are CS- note on AE form	

Venepuncture PRE 1st ETT

Samples required:

- 3x EDTA (purple 4ml) – FBC, BNP, storage
- 2x clotted (yellow 5ml) – UE/LFT's, storage

V6B	Blood samples taken for FBC/UE/LFT/BNP/storage	

ETT 1-hour pre study drug stat dose

Resting ECG

Normal / Abnormal (circle one)

State any abnormalities: _____

ETT Targets

Target exercise time for age: ___min : ___sec

Target HR for age: ____ bpm

Results

Max HR achieved: ____ bpm

% of target HR achieved: _____ %

Max workload in METS: _____

Stage at ST depression: (1-5) ____

Total exercise time _____

ECG Interpretation

ECG changes (circle one): yes / no

ST depression (circle one) yes / no amount: _____

ST depression >1 mm onset @ ___min : ___sec

Leads with ST changes: _____

Time of onset of other ECG changes: ___min : ___sec

ST elevation (circle one) yes / no amount: _____

Arrhythmias (circle one): yes / no

ALL ACS Subject ID .... Initials ..... Date: __ /__ /____

Type (circle one or more):
CHB / Asystole / VT / VF / Change in morphology
Other _____
Chest Pain
Chest pain (circle one): yes / no
Time to chest pain (if present): ___min : ___sec

Reasons for stopping the treadmill:

- ❑ ST depression >1 mm
- ❑ Other ECG changes, specify reason_____
- ❑ Chest Pain
- ❑ Shortness of breath
- ❑ Leg pain/leg tiredness
- ❑ Hypotension
- ❑ Hypertension
- ❑ Other, specify _____

BP Record

Start: / mmHg	9min: / mmHg
3min: / mmHg	12min: / mmHg
6min: / mmHg	End: / mmHg

V6C Baseline ETT 1 hour pre study drug dosing completed ☐

Medication Issued (Stat Dose)

Study medication supply

Randomisation Number _____

Date and time of stat dose administered _____

Comments _____

_____

_____

V6D Stat dose Medication checked ☐

Venepuncture 2 hour post study drug

Samples required:

- 1 x clotted (yellow 5ml) uric acid storage

V6E Blood samples 2 hour post study drug for Uric acid ☐

ALL ACS Subject ID .... Initials ..... Date: __ /__ /____

Visit 6

Venepuncture 4 hour post study drug (PRE 2nd ETT)

Samples required:

- 1 x clotted (yellow 5ml) uric acid, Hs CRP, Troponin
- 2 x EDTA (purple) BNP, oxidised LDL, Isoprostane

V6F	Blood samples 4 hour post study drug for BNP/ Storage	

Visit 6

ETT 2: Four-hour post study drug stat dose

Resting ECG

Normal / Abnormal (circle one)

State any abnormalities: _____

ETT Targets

Target exercise time for age: ___min : ___sec

Target HR for age: _____ bpm

Results

Max HR achieved: _____ bpm

% of target HR achieved: _____ %

Max workload in METS: _____

Stage at ST depression: (1-5) _____

Total exercise time _____

ECG Interpretation

ECG changes (circle one): yes / no

ST depression (circle one) yes / no amount: _____

ST depression >1 mm onset @ ___min : ___sec

Leads with ST changes: _____

Time of onset of other ECG changes: ___min : ___sec

ST elevation (circle one) yes / no amount: _____

Arrhythmias (circle one): yes / no

Type (circle one or more):

CHB / Asystole / VT / VF / Change in morphology

Other _____

Chest Pain

Chest pain (circle one): yes / no

Time to chest pain (if present): ___min : ___sec

Visit 6

Reasons for stopping the treadmill:

- ❑ ST depression >1 mm
- ❑ Other ECG changes, specify reason_____
- ❑ Chest Pain
- ❑ Shortness of breath
- ❑ Leg pain/leg tiredness
- ❑ Hypotension
- ❑ Hypertension
- ❑ Other, specify _____

BP Record

Start: / mmHg	9min: / mmHg
3min: / mmHg	12min: / mmHg
6min: / mmHg	End: / mmHg

V6G ETT 4 hour post study drug dosing completed ☐

- 1 x EDTA (purple) BNP
- 1 x clotted (yellow 5ml) uric acid, Troponin

V6H Post 2nd ETT bloods – uric acid, BNP, Troponin ☐

V6I Adverse Events recorded ☐

Study medication supply

Number of tablets supplied _____

Randomisation Number _____

V6J Bottle of study medication issued, complete with instructions ☐

V6k	Angina Diary issued, complete with instructions	

Final Visit 6 checks

V6L	Date for next visit booked and recorded on front of CRF	

Signed		Name		Date	

ALL ACS Subject ID .... Initials ..... Date: __ /__ /____

Visit 7

Visit 7 (Day 13 – Progress Visit)

Visit 6 safety bloods results: **CS/NCS (circle)**

V7A Visit 6 blood results documented: any that are CS- note on AE form	

Venepuncture PRE ETT

Samples required:

- 3 x EDTA (purple 4ml) – FBC, BNP, oxidised LDL, Isoprostane
- 2x clotted (yellow 5ml) – UE/LFT, hs CRP, Troponin

V7B Blood samples taken for FBC/U&Es/LFT/BNP/Troponin/hsCRP/isoprostane/oxidised LDL	

Resting ECG

Normal / Abnormal (circle one)

State any abnormalities: _____

ETT Targets

Target exercise time for age: ___min : ___sec

Target HR for age: ____ bpm

Results

Max HR achieved: ____ bpm

% of target HR achieved: _____ %

Max workload in METS: _____

Stage at ST depression: (1-5) ____

Total exercise time _____

ECG Interpretation

ECG changes (circle one): yes / no

ST depression (circle one) yes / no amount: _____

ST depression >1 mm onset @ ___min : ___sec

Leads with ST changes: _____

Time of onset of other ECG changes: ___min : ___sec

ST elevation (circle one) yes / no amount: _____

Arrhythmias (circle one): yes / no

Type (circle one or more):

CHB / Asystole / VT / VF / Change in morphology

Other _____

ALL ACS Subject ID .... Initials ..... Date: __/__/____

Chest Pain

Chest pain (circle one): yes / no

Time to chest pain (if present): ___min : ___sec

Reasons for stopping the treadmill:

❑ ST depression >1 mm

❑ Other ECG changes, specify reason_____

❑ Chest Pain

❑ Shortness of breath

❑ Leg pain/leg tiredness

❑ Hypotension

❑ Hypertension

❑ Other, specify _____

BP Record

Start: / mmHg	9min: / mmHg
3min: / mmHg	12min: / mmHg
6min: / mmHg	End: / mmHg

Samples required:

- 2 x EDTA (purple 4ml) BNP, oxidised LDL, Isoprostane
- 1x clotted (yellow 5ml) hs CRP, Troponin

V7C	**Blood samples post ETT for BNP/hsCRP/Troponin/isoprostane/oxidised LDL**	

Medication compliance

Number of tablets remaining _____

Comments _____

_____

V7D	**Medication compliance checked**	
V7E	**Adverse event log updated**	
V7F	**Angina Diary; checked**	
V7G	**Medications log updated**	

ALL ACS Subject ID .... Initials ..... Date: __ /__ /____

Final Visit 7 checks

V7H	**Date for next visit booked and recorded on front of CRF**	

Signed		**Name**		**Date**	

ALL ACS Subject ID .... Initials ..... Date: __ /__ /____

Visit 8

Visit 8 (Day 17 – Progress Visit)

Visit 7 safety bloods results: **CS/NCS (circle)**

V8A	**Visit 7 blood results documented: any that are CS- note on AE form**	

Venepuncture PRE ETT

Samples required:

- 2x EDTA (purple 4ml) – FBC
- 3x clotted (yellow 5ml) – UE/LFT, storage

V8B	**Blood samples taken for FBC/U&Es/LFT/BNP/storage**	

Resting ECG

Normal / Abnormal (circle one)

State any abnormalities: _____

ETT Targets

Target exercise time for age: ___min : ___sec

Target HR for age: ____ bpm

Results

Max HR achieved: ____ bpm

% of target HR achieved: _____ %

Max workload in METS: _____

Stage at ST depression: (1-5) ____

Total exercise time _____

ECG Interpretation

ECG changes (circle one): yes / no

ST depression (circle one) yes / no amount: _____

ST depression >1 mm onset @ ___min : ___sec

Leads with ST changes: _____

Time of onset of other ECG changes: ___min : ___sec

ST elevation (circle one) yes / no amount: _____

Arrhythmias (circle one): yes / no

Type (circle one or more):

CHB / Asystole / VT / VF / Change in morphology

Other _____

ALL ACS Subject ID .... Initials ..... Date: __/__/____

Chest Pain

Chest pain (circle one): yes / no

Time to chest pain (if present): ___min : ___sec

Reasons for stopping the treadmill:

- ❑ ST depression >1 mm
- ❑ Other ECG changes, specify reason_____
- ❑ Chest Pain
- ❑ Shortness of breath
- ❑ Leg pain/leg tiredness
- ❑ Hypotension
- ❑ Hypertension
- ❑ Other, specify _____

BP Record

Start: / mmHg	9min: / mmHg
3min: / mmHg	12min: / mmHg
6min: / mmHg	End: / mmHg

Venepuncture 2 hour post study drug

Samples required:

- 1 x clotted (yellow 5ml) uric acid /Troponin

V8C	**Blood samples post ETT for Uric Acid/Troponin**	

Medication compliance

Number of tablets remaining _____

Comments _____

_____

V8D	**Medication compliance checked & Unused medications returned to store**	
V8E	**Adverse event log updated**	
V8F	**Angina Diary; checked**	
V8G	**Medications log updated**	

Final Visit 8 checks

ALL ACS Subject ID .... Initials ..... Date: __ /__ /____

V8H	Date for next visit booked and recorded on front of CRF	

Signed		Name		Date	

Visit 9

ALL ACS Subject ID .... Initials ..... Date: __/__/____

Visit 9 Randomisation Visit (Day 24)

Visit 8 safety bloods results: **CS/NCS (circle)**

V9A	**Visit 8 blood results documented: any that are CS- note on AE form**	

Venepuncture PRE 1st ETT

Samples required:

- 3x EDTA (purple 4ml) FBC, Storage bloods
- 2x clotted (yellow 5ml) UE/LFT's, BNP, storage bloods

V9B	**Blood samples taken for FBC/UE&LFT/BNP/Storage**	

ETT 1: One-hour pre study drug stat dose

Resting ECG

Normal / Abnormal (circle one)

State any abnormalities: _____

ETT Targets

Target exercise time for age: ___min : ___sec

Target HR for age: ____ bpm

Results

Max HR achieved: ____ bpm

% of target HR achieved: _____ %

Max workload in METS: _____

Stage at ST depression: (1-5) ____

Total exercise time _____

ECG Interpretation

ECG changes (circle one): yes / no

ST depression (circle one) yes / no amount: _____

ST depression >1 mm onset @ ___min : ___sec

Leads with ST changes: _____

Time of onset of other ECG changes: ___min : ___sec

ST elevation (circle one) yes / no amount: _____

Arrhythmias (circle one): yes / no

Type (circle one or more):

CHB / Asystole / VT / VF / Change in morphology

Other _____

ALL ACS Subject ID .... Initials ..... Date: __ /__ /____

Visit 9

Chest Pain

Chest pain (circle one): yes / no

Time to chest pain (if present): ___min : ___sec

Reasons for stopping the treadmill:

- ❑ ST depression >1 mm
- ❑ Other ECG changes, specify reason_____
- ❑ Chest Pain
- ❑ Shortness of breath
- ❑ Leg pain/leg tiredness
- ❑ Hypotension
- ❑ Hypertension
- ❑ Other, specify _____

BP Record

Start: / mmHg		9min: / mmHg	
3min: / mmHg		12min: / mmHg	
6min: / mmHg		End: / mmHg	

V9C Baseline ETT 1 hour pre study drug dosing completed ☐

Medication Issued (Stat Dose)

Study medication supply

Randomisation Number _____

Date and time of stat dose administered _____

Comments _____

V9D Stat dose Medication checked ☐

Venepuncture 2 hour post study drug

Samples required:

- x clotted (yellow 5ml) uric acid storage

V9E Blood samples 2 hour post study drug for Uric acid ☐

Venepuncture 4 hour post study drug (PRE 2nd ETT)

Samples required:

- 1 x clotted (yellow 5ml) uric acid, Hs CRP, Troponin
- 2 x EDTA (purple) BNP, oxidised LDL, Isoprostane

ALL ACS Subject ID .... Initials ..... Date: __ /__ /____

V9F	Blood samples 4 hour post study drug (pre 2nd ETT) Research Bloods		Visit 9

ETT 2: Four-hour post study drug stat dose

Resting ECG

Normal / Abnormal (circle one)

State any abnormalities: _____

ETT Targets

Target exercise time for age: ___min : ___sec

Target HR for age: ____ bpm

Results

Max HR achieved: ____ bpm

% of target HR achieved: _____ %

Max workload in METS: _____

Stage at ST depression: (1-5) ____

Total exercise time _____

ECG Interpretation

ECG changes (circle one): yes / no

ST depression (circle one) yes / no amount: _____

ST depression >1 mm onset @ ___min : ___sec

Leads with ST changes: _____

Time of onset of other ECG changes: ___min : ___sec

ST elevation (circle one) yes / no amount: _____

Arrhythmias (circle one): yes / no

Type (circle one or more):

CHB / Asystole / VT / VF / Change in morphology

Other _____

Chest Pain

Chest pain (circle one): yes / no

Time to chest pain (if present): ___min : ___sec

ALL ACS Subject ID .... Initials ..... Date: __ /__ /____

Visit 9

Reasons for stopping the treadmill:

- ❑ ST depression >1 mm
- ❑ Other ECG changes, specify reason_____
- ❑ Chest Pain
- ❑ Shortness of breath
- ❑ Leg pain/leg tiredness
- ❑ Hypotension
- ❑ Hypertension
- ❑ Other, specify _____

BP Record

Start: / mmHg	9min: / mmHg
3min: / mmHg	12min: / mmHg
6min: / mmHg	End: / mmHg

V9G	**ETT 4 hour post study drug dosing completed**	

- 1 x EDTA (purple) BNP
- 1 x clotted (yellow 5ml) uric acid, Troponin

V9H	**Post 2nd ETT bloods – uric acid, BNP, Troponin**	

V9I	**Adverse Events recorded**	

Study medication supply

Number of tablets supplied _____

Randomisation Number _____

V9J	**Bottle of study medication issued, complete with instructions**	

V9K	**Angina Diary issued, complete with instructions**	

Final Visit 9 checks

V9L	**Date for next visit booked and recorded on front of CRF**	

Signed		Name		Date	

Visit 10

ALL ACS Subject ID .... Initials ..... Date: __ /__ /____

Visit 10 (Day 25 – Progress Visit)

Visit 9 safety bloods results: **CS/NCS (circle)**

V10A	**Visit 9 blood results documented: any that are CS- note on AE form**	

Venepuncture PRE ETT

Samples required:

- 3 x EDTA (purple 4ml) – FBC, BNP, oxidised LDL, Isoprostane
- 2x clotted (yellow 5ml) – UE/LFT, hs CRP, Troponin

V10B	**Blood samples taken for FBC/U&Es/LFT/BNP/storage bloods**	

Resting ECG

Normal / Abnormal (circle one)

State any abnormalities: _____

ETT Targets

Target exercise time for age: ___min : ___sec

Target HR for age: ____ bpm

Results

Max HR achieved: ____ bpm

% of target HR achieved: _____ %

Max workload in METS: _____

Stage at ST depression: (1-5) ____

Total exercise time _____

ECG Interpretation

ECG changes (circle one): yes / no

ST depression (circle one) yes / no amount: _____

ST depression >1 mm onset @ ___min : ___sec

Leads with ST changes: _____

Time of onset of other ECG changes: ___min : ___sec

ST elevation (circle one) yes / no amount: _____

Arrhythmias (circle one): yes / no

Type (circle one or more):

CHB / Asystole / VT / VF / Change in morphology

Other _____

ALL ACS Subject ID .... Initials ..... Date: __/__/____

Visit 10

Chest Pain

Chest pain (circle one): yes / no

Time to chest pain (if present): ___min : ___sec

Reasons for stopping the treadmill:

- ❑ ST depression >1 mm
- ❑ Other ECG changes, specify reason_____
- ❑ Chest Pain
- ❑ Shortness of breath
- ❑ Leg pain/leg tiredness
- ❑ Hypotension
- ❑ Hypertension
- ❑ Other, specify _____

BP Record

Start: / mmHg		9min: / mmHg	
3min: / mmHg		12min: / mmHg	
6min: / mmHg		End: / mmHg	

Venepuncture 2 hour post study drug

Samples required:

- 2 x EDTA (purple 4ml) BNP, oxidised LDL, Isoprostane
- 1x clotted (yellow 5ml) hs CRP, Troponin

V10C Blood samples post ETT for BNP/hsCRP/Troponin/isoprostane/oxidised LDL	

Medication compliance

Number of tablets remaining _____

Comments _____

_____

V10D Medication compliance checked	
V10E Adverse event log updated	
V10F Angina Diary; checked	
V10G Medications log updated	

ALL ACS Subject ID .... Initials ..... Date: __/__/____

Final Visit 10 checks

V10H Date for next visit booked and recorded on front of CRF	

Signed		**Name**		**Date**	

Visit 11

ALL ACS Subject ID .... Initials ..... Date: __ /__ /____

Visit 11 (Day 29) FINAL Visit

Visit 10 safety bloods results: **CS/NCS (circle)**

V11A Visit 10 blood results documented: any that are CS- note on AE form	

Venepuncture PRE ETT

Samples required:

- 3x EDTA (purple 4ml) – FBC, storage bloods
- 2x clotted (yellow 5ml) – UE/LFT/BNP/storage

V11B Blood samples taken for FBC/U&Es/LFT/BNP/ uric acid/Troponin/hsCRP/isoprostane/oxidised LDL	

Resting ECG

Normal / Abnormal (circle one)

State any abnormalities: _____

ETT Targets

Target exercise time for age: ___min : ___sec

Target HR for age: ____ bpm

Results

Max HR achieved: ____ bpm

% of target HR achieved: _____ %

Max workload in METS: _____

Stage at ST depression: (1-5) ____

Total exercise time _____

Time to chest pain _____

ECG Interpretation

ECG changes (circle one): yes / no

ST depression (circle one) yes / no amount: _____

ST depression >1 mm onset @ ___min : ___sec

Leads with ST changes: _____

Time of onset of other ECG changes: ___min : ___sec

ST elevation (circle one) yes / no amount: _____

Arrhythmias (circle one): yes / no

Type (circle one or more):

CHB / Asystole / VT / VF / Change in morphology

Other _____

ALL ACS Subject ID .... Initials ..... Date: __ /__ /____

Visit 11

Chest Pain

Chest pain (circle one): yes / no

Time to chest pain (if present): ___min : ___sec

Reasons for stopping the treadmill:

- ❑ ST depression >1 mm
- ❑ Other ECG changes, specify reason_____
- ❑ Chest Pain
- ❑ Shortness of breath
- ❑ Leg pain/leg tiredness
- ❑ Hypotension
- ❑ Hypertension
- ❑ Other, specify _____

BP Record

Start: / mmHg		9min: / mmHg	
3min: / mmHg		12min: / mmHg	
6min: / mmHg		End: / mmHg	

Venepuncture 2 hour post study drug

Samples required:

- 2 x EDTA (purple 4ml) BNP, oxidised LDL, Isoprostane
- 1x clotted (yellow 5ml) hs CRP, Troponin

V11C Blood samples post ETT for BNP/hsCRP/Troponin/isoprostane/oxidised LDL	

Number of tablets remaining _____

Comments _____

_____

V11D Medication compliance checked & Unused medications returned to store	
V11E Adverse event log updated	
V11F Angina Diary; checked	
V11G Medications log updated	

ALL ACS Subject ID .... Initials ..... Date: __ /__ /____

V11H	Completion of study form (on next page) filled out	

Signed		Name		Date	

ALL ACS Subject ID .... Initials ..... Date: __/__/____

Completion of Study/Early Withdrawal Form

Completion

Did the subject complete the study? Yes ❑ No ❑

Date of completion/withdrawal: ____/____/_____

If subject did not complete, give reason:

_____

_____

_____

_____

Follow-up

Is any follow-up required? Yes ❑ No ❑

If so, provide details:

_____

_____

_____

Protocol

Were there any deviations from protocol? Yes ❑ No ❑

If so, provide details and note in Trial Master File: Protocol Deviation Log:

_____

_____

_____

Signed		Name		Date	

ALL ACS Subject ID .... Initials ..... Date: __/__/____

Medications Log

Medication	*Indication*	*Dose*	*Frequency*	*Route*	*Start date (if known)*	*End date (if discontinued)*

ALL ACS Subject ID .... Initials ..... Date: __ /__ /____

Safety Blood Results

	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11
Hb											
Wcc											
Plts											
Na											
K											
Urea											
Creat											
ALT											
Bili											
Alk phos											
Alb											

ALL ACS Subject ID .... Initials ..... Date: __ /__ /____

Safety Blood Results

	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11
Hb											
Wcc											
Plts											
Na											
K											
Urea											
Creat											
ALT											
Bili											
Alk phos											
Alb											

ALL ACS Subject ID .... Initials ..... Date: __/__/____

Research Blood results – record BNP on day- rest will be populated at end of study

	Visit 3 Pre 1st Dose ETT	Visit 3 2 hour Post dose	Visit 3 Pre 4 hour ETT	Visit 3 Post 4 hour ETT	Visit 4 Pre ETT	Visit 4 Post ETT	Visit 5 Pre ETT	Visit 5 Post ETT
Date								
Time								
BNP (pg/ml)								
Uric Acid (mmol/l)								
Troponin (ng/ml)								
hsCRP (mg/l)								
Isoprostanes (pg/ml)								
Oxidised LDL (U/l)								

Do not complete shaded areas on results form.

ALL ACS Subject ID .... Initials ..... Date: __/__/____

Research Blood results – record BNP on day- rest will be populated at end of study

	Visit 6 Pre 1st Dose ETT	Visit 6 2 hour Post dose	Visit 6 Pre 4 hour ETT	Visit 6 Post 4 hour ETT	Visit 7 Pre ETT	Visit 7 Post ETT	Visit 8 Pre ETT	Visit 8 Post ETT
Date								
Time								
BNP (pg/ml)								
Uric Acid (mmol/l)								
hsTroponin T (ng/ml)								
hsCRP (mg/l)								
Isoprostanes (pg/ml)								
Oxidised LDL (U/l)								

Do not complete shaded areas on results form.

ALL ACS Subject ID .... Initials ..... Date: __/__/____

Research Blood results – record BNP on day- rest will be populated at end of study

	Visit 9 Pre 1st Dose ETT	Visit 9 2 hour Post dose	Visit 9 Pre 4 hour ETT	Visit 9 Post 4 hour ETT	Visit 10 Pre ETT	Visit 10 Post ETT	Visit 11 Pre ETT	Visit 11 Post ETT
Date								
Time								
BNP (pg/ml)								
Uric Acid (mmol/l)								
hsTroponin T (ng/ml)								
hsCRP (mg/l)								
Isoprostanes (pg/ml)								
Oxidised LDL (U/l)								

Do not complete shaded areas on results form.

ALL ACS Subject ID ..... Initials .....

Adverse Events Log

Description of adverse event (provide additional information on notes pages if required)	**Date of onset**	**Date reported**	**Severity** 1. Mild 2. Moderate 3. Severe	**Relationship to IMP** 1. Unrelated 2. Possible 3. Probable 4. Definite 5. Suspected 6. Unknown	**Action taken** 1. None 2. IMP dose reduced/temp withheld 3. IMP stopped 4. Con. meds commenced 5. Other	**Dat** (if n end as o

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H Angina Log

ANGINA LOG

Angina is pain or discomfort in the chest that occurs when your heart doesn't get enough oxygen. A log (or record) of your angina symptoms helps show what angina is like for you and how your angina pattern changes over time. The log helps your doctor regulate your medicines and decide on future treatments.

American Heart Association
Learn and Live

- On days you have angina, fill in the date and the number of times you had angina that day.
- Write down what triggered your angina, if anything. Common triggers are exercise, emotions, eating a large meal and going out in cold weather. If there was no trigger, write "no trigger."
- Use a scale of 1 to 4 to rate your pain or discomfort:
  1 = mild, 2 = somewhat strong, 3 = severe, 4 = very severe.
- Note how long the angina lasted and what you did for it (such as rest or take nitroglycerin).
- Take this sheet with you and show it to your doctor at each visit.

DATE	NUMBER OF ANGINA ATTACKS	TRIGGER	RATING (1-4)	HOW LONG IT LASTED	WHAT DID YOU DO FOR IT?
example JUNE 16	example 1	example big meal	example 2	example 1 minute	example nitro

www.AmericanHeart.org/CardiacRehab

6 References

1. Management of stable angina. Nice clinical guideline 73. November 2003.
2. Management of stable angina. Scottish Intercollegiate Guidelines Network. 2007.
3. Guidelines on the management of stable angina pectoris: full text. Eur Heart J. 2006.
4. Rajagopalan S, Meng XP, Ramasamy S, Harrison DG, Galis ZS. Reactive oxygen species produced by macrophage-derived foam cells regulate the activity of vascular matrix metalloproteinases in vitro. Implications for atherosclerotic plaque stability. J Clin Invest. 1996 Dec 1;98(11):2572-9.
5. Gibbons RJ, Balady GJ, Bricker JT, Chaitman BR, Fletcher GF, Froelicher VF, et al. ACC/AHA 2002 guideline update for exercise testing: summary article: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee to Update the 1997 Exercise Testing Guidelines). Circulation. 2002 Oct 1;106(14):1883-92.
6. Kwok Y, Kim C, Grady D, Segal M, Redberg R. Meta-analysis of exercise testing to detect coronary artery disease in women. Am J Cardiol. 1999 Mar 1;83(5):660-6.
7. Chest pain of recent onset: Assessment and diagnosis of recent onset chest pain or discomfort of suspected cardiac origin. Nice clinical guideline CG95. March 2010.
8. PJ. P. Pathophysiology and clinical presentation of ischaemic chest pain. wwwuptodatecom. 2011.
9. Nesto RW, Kowalchuk GJ. The ischemic cascade: temporal sequence of hemodynamic, electrocardiographic and symptomatic expressions of ischemia. Am J Cardiol. 1987 Mar 9;59(7):23C-30C.
10. Bailey TG, Perissiou M, Windsor M, Russell F, Golledge J, Green DJ, et al. Cardiorespiratory fitness modulates the acute flow-mediated dilation response following high-intensity but not moderate-intensity exercise in elderly men. J Appl Physiol (1985). 2017 May 01;122(5):1238-48.
11. Crea F, Gaspardone A. New look to an old symptom: angina pectoris. Circulation. 1997 Nov 18;96(10):3766-73.
12. Foreman RD. Mechanisms of cardiac pain. Annu Rev Physiol. 1999;61:143-67.
13. Longhurst JC, Tjen ALSC, Fu LW. Cardiac sympathetic afferent activation provoked by myocardial ischemia and reperfusion. Mechanisms and reflexes. Ann N Y Acad Sci. 2001 Jun;940:74-95.
14. Cohn PF, Fox KM, Daly C. Silent myocardial ischemia. Circulation. 2003 Sep 9;108(10):1263-77.
15. Acute Coronary Syndromes. Scottish Intercollegiate Guidelines Network. 2007.
16. Otsuka F KF, Virmani R. The role of the vulnerable plaque in acute coronary syndromes. wwwuptodatecom. 2012.
17. Day RO, Graham GG, Hicks M, McLachlan AJ, Stocker SL, Williams KM. Clinical pharmacokinetics and pharmacodynamics of allopurinol and oxypurinol. Clin Pharmacokinet. 2007;46(8):623-44.
18. George J, Struthers AD. Role of urate, xanthine oxidase and the effects of allopurinol in vascular oxidative stress. Vasc Health Risk Manag. 2009;5(1):265-72.
19. Kelkar A, Kuo A, Frishman WH. Allopurinol as a cardiovascular drug. Cardiol Rev. 2011 Nov-Dec;19(6):265-71.
20. Harrison R. Structure and function of xanthine oxidoreductase: where are we now? Free Radic Biol Med. 2002 Sep 15;33(6):774-97.
21. Alderman MH. Uric acid and cardiovascular risk. Curr Opin Pharmacol. 2002 Apr;2(2):126-30.
22. Kohn PM, Prozan GB. Hyperuricemia; relationship to hypercholesteremia and acute myocardial infarction. J Am Med Assoc. 1959 Aug 15;170(16):1909-12.

23. Beard JT, 2nd. Serum uric acid and coronary heart disease. Am Heart J. 1983 Aug;106(2):397-400.
24. Fang J, Alderman MH. Serum uric acid and cardiovascular mortality the NHANES I epidemiologic follow-up study, 1971-1992. National Health and Nutrition Examination Survey. JAMA. 2000 May 10;283(18):2404-10.
25. Okura T, Higaki J, Kurata M, Irita J, Miyoshi K, Yamazaki T, et al. Elevated serum uric acid is an independent predictor for cardiovascular events in patients with severe coronary artery stenosis: subanalysis of the Japanese Coronary Artery Disease (JCAD) Study. Circ J. 2009 May;73(5):885-91.
26. Zoppini G, Targher G, Negri C, Stoico V, Perrone F, Muggeo M, et al. Elevated serum uric acid concentrations independently predict cardiovascular mortality in type 2 diabetic patients. Diabetes Care. 2009 Sep;32(9):1716-20.
27. Ioachimescu AG, Brennan DM, Hoar BM, Kashyap SR, Hoogwerf BJ. Serum uric acid, mortality and glucose control in patients with Type 2 diabetes mellitus: a PreCIS database study. Diabet Med. 2007 Dec;24(12):1369-74.
28. Grayson PC, Kim SY, LaValley M, Choi HK. Hyperuricemia and incident hypertension: a systematic review and meta-analysis. Arthritis Care Res (Hoboken). 2011 Jan;63(1):102-10.
29. Hoieggen A, Alderman MH, Kjeldsen SE, Julius S, Devereux RB, De Faire U, et al. The impact of serum uric acid on cardiovascular outcomes in the LIFE study. Kidney Int. 2004 Mar;65(3):1041-9.
30. Niskanen LK, Laaksonen DE, Nyyssonen K, Alfthan G, Lakka HM, Lakka TA, et al. Uric acid level as a risk factor for cardiovascular and all-cause mortality in middle-aged men: a prospective cohort study. Arch Intern Med. 2004 Jul 26;164(14):1546-51.
31. Culleton BF, Larson MG, Kannel WB, Levy D. Serum uric acid and risk for cardiovascular disease and death: the Framingham Heart Study. Ann Intern Med. 1999 Jul 6;131(1):7-13.
32. Moriarity JT, Folsom AR, Iribarren C, Nieto FJ, Rosamond WD. Serum uric acid and risk of coronary heart disease: Atherosclerosis Risk in Communities (ARIC) Study. Ann Epidemiol. 2000 Apr;10(3):136-43.
33. Jee SH, Lee SY, Kim MT. Serum uric acid and risk of death from cancer, cardiovascular disease or all causes in men. Eur J Cardiovasc Prev Rehabil. 2004 Jun;11(3):185-91.
34. Poullis M. Serum uric acid and cardiovascular disease risk. Ann Intern Med. 2000 Apr 4;132(7):591-2.
35. Hashemi M, Yavari M, Amiri N, Taheri H, Shaigannia I, Moghadas L, et al. Uric acid: a risk factor for coronary atherosclerosis? Cardiovasc J S Afr. 2007 Jan-Feb;18(1):16-9.
36. Persky VW, Dyer AR, Idris-Soven E, Stamler J, Shekelle RB, Schoenberger JA, et al. Uric acid: a risk factor for coronary heart disease? Circulation. 1979 May;59(5):969-77.
37. Bos MJ, Koudstaal PJ, Hofman A, Witteman JC, Breteler MM. Uric acid is a risk factor for myocardial infarction and stroke: the Rotterdam study. Stroke. 2006 Jun;37(6):1503-7.
38. Rich MW. Uric acid: is it a risk factor for cardiovascular disease? Am J Cardiol. 2000 Apr 15;85(8):1018-21.
39. George J, Carr E, Davies J, Belch JJ, Struthers A. High-dose allopurinol improves endothelial function by profoundly reducing vascular oxidative stress and not by lowering uric acid. Circulation. 2006 Dec 5;114(23):2508-16.
40. Baldus S, Koster R, Chumley P, Heitzer T, Rudolph V, Ostad MA, et al. Oxypurinol improves coronary and peripheral endothelial function in patients with coronary artery disease. Free Radic Biol Med. 2005 Nov 1;39(9):1184-90.

41. Sies H, Cadenas E. Oxidative stress: damage to intact cells and organs. Philos Trans R Soc Lond B Biol Sci. 1985 Dec 17;311(1152):617-31.
42. MacNicol JL, Lindinger MI, Pearson W. A Time Course Evaluation of Inflammatory and Oxidative Markers Following High Intensity Exercise in Horses: a Pilot Study. J Appl Physiol (1985). 2017 Oct 26:jap 00461 2017.
43. Rajendra NS, Ireland S, George J, Belch JJ, Lang CC, Struthers AD. Mechanistic insights into the therapeutic use of high-dose allopurinol in angina pectoris. J Am Coll Cardiol. 2011 Aug 16;58(8):820-8.
44. Noman A, Ang DS, Ogston S, Lang CC, Struthers AD. Effect of high-dose allopurinol on exercise in patients with chronic stable angina: a randomised, placebo controlled crossover trial. Lancet. 2010 Jun 19;375(9732):2161-7.
45. Wright DF, Stamp LK, Merriman TR, Barclay ML, Duffull SB, Holford NH. The population pharmacokinetics of allopurinol and oxypurinol in patients with gout. Eur J Clin Pharmacol. 2013 Jul;69(7):1411-21.
46. Levinger I, Shaw CS, Stepto NK, Cassar S, McAinch AJ, Cheetham C, et al. What Doesn't Kill You Makes You Fitter: A Systematic Review of High-Intensity Interval Exercise for Patients with Cardiovascular and Metabolic Diseases. Clin Med Insights Cardiol. 2015;9:53-63.
47. Halevy S, Ghislain PD, Mockenhaupt M, Fagot JP, Bouwes Bavinck JN, Sidoroff A, et al. Allopurinol is the most common cause of Stevens-Johnson syndrome and toxic epidermal necrolysis in Europe and Israel. J Am Acad Dermatol. 2008 Jan;58(1):25-32.
48. Simons M LR. New therapies for angina pectoris. wwwuptodatecom. 2011.
49. George J, Struthers A. The OPT-CHF (Oxypurinol Therapy for Congestive Heart Failure) trial: a question of dose. J Am Coll Cardiol. 2009 Jun 23;53(25):2405.
50. Kao MP, Ang DS, Gandy SJ, Nadir MA, Houston JG, Lang CC, et al. Allopurinol benefits left ventricular mass and endothelial dysfunction in chronic kidney disease. J Am Soc Nephrol. 2011 Jul;22(7):1382-9.
51. Cappola TP, Kass DA, Nelson GS, Berger RD, Rosas GO, Kobeissi ZA, et al. Allopurinol improves myocardial efficiency in patients with idiopathic dilated cardiomyopathy. Circulation. 2001 Nov 13;104(20):2407-11.
52. Ekelund UE, Harrison RW, Shokek O, Thakkar RN, Tunin RS, Senzaki H, et al. Intravenous allopurinol decreases myocardial oxygen consumption and increases mechanical efficiency in dogs with pacing-induced heart failure. Circ Res. 1999 Sep 3;85(5):437-45.
53. Cappola AR, Bandeen-Roche K, Wand GS, Volpato S, Fried LP. Association of IGF-I levels with muscle strength and mobility in older women. J Clin Endocrinol Metab. 2001 Sep;86(9):4139-46.
54. Farquharson CA, Butler R, Hill A, Belch JJ, Struthers AD. Allopurinol improves endothelial dysfunction in chronic heart failure. Circulation. 2002 Jul 9;106(2):221-6.
55. Butler R, Morris AD, Belch JJ, Hill A, Struthers AD. Allopurinol normalizes endothelial dysfunction in type 2 diabetics with mild hypertension. Hypertension. 2000 Mar;35(3):746-51.
56. Guthikonda S, Sinkey C, Barenz T, Haynes WG. Xanthine oxidase inhibition reverses endothelial dysfunction in heavy smokers. Circulation. 2003 Jan 28;107(3):416-21.
57. Halvorsen B, Otterdal K, Dahl TB, Skjelland M, Gullestad L, Oie E, et al. Atherosclerotic plaque stability--what determines the fate of a plaque? Prog Cardiovasc Dis. 2008 Nov-Dec;51(3):183-94.
58. Santos CC, Diniz TA, Inoue DS, Gerosa-Neto J, Panissa VL, Pimentel GD, et al. Influence to high-intensity intermittent and moderate-intensity continuous exercise on indices of cardio-inflammatory health in men. J Exerc Rehabil. 2016 Dec;12(6):618-23.

59. Davies MJ. Reactive oxygen species, metalloproteinases, and plaque stability. Circulation. 1998 Jun 23;97(24):2382-3.
60. Morgan JP. Cellular Mechanisms of Diastolic Dysfunction

wwwuptodatecom. 2014.
61. Khatib SY, Farah H, El-Migdadi F. Allopurinol enhances adenine nucleotide levels and improves myocardial function in isolated hypoxic rat heart. Biochemistry (Mosc). 2001 Mar;66(3):328-33.
62. Hirsch GA, Bottomley PA, Gerstenblith G, Weiss RG. Allopurinol Acutely Increases Adenosine Triphospate Energy Delivery in Failing Human Hearts. J Am Coll Cardiol. 2012 Feb 28;59(9):802-8.
63. Mellin V, Isabelle M, Oudot A, Vergely-Vandriesse C, Monteil C, Di Meglio B, et al. Transient reduction in myocardial free oxygen radical levels is involved in the improved cardiac function and structure after long-term allopurinol treatment initiated in established chronic heart failure. Eur Heart J. 2005 Aug;26(15):1544-50.
64. Rentoukas E, Tsarouhas K, Tsitsimpikou C, Lazaros G, Deftereos S, Vavetsi S. The prognostic impact of allopurinol in patients with acute myocardial infarction undergoing primary percutaneous coronary intervention. Int J Cardiol. 2010 Nov 19;145(2):257-8.
65. Engberding N, Spiekermann S, Schaefer A, Heineke A, Wiencke A, Muller M, et al. Allopurinol attenuates left ventricular remodeling and dysfunction after experimental myocardial infarction: a new action for an old drug? Circulation. 2004 Oct 12;110(15):2175-9.
66. Montuschi P, Barnes PJ, Roberts LJ, 2nd. Isoprostanes: markers and mediators of oxidative stress. FASEB J. 2004 Dec;18(15):1791-800.
67. Givertz MM. Role of oxidative streses in heart failure. wwwuptodatecom. 2012.
68. Meisinger C, Baumert J, Khuseyinova N, Loewel H, Koenig W. Plasma oxidized low-density lipoprotein, a strong predictor for acute coronary heart disease events in apparently healthy, middle-aged men from the general population. Circulation. 2005 Aug 2;112(5):651-7.
69. Ghosh J, Mishra TK, Rao YN, Aggarwal SK. Oxidised LDL, HDL cholesterol, LDL cholesterol levels in patients of coronary artery disease. Indian J Clin Biochem. 2006 Mar;21(1):181-4.
70. Tsimikas S, Witztum JL. Measuring circulating oxidized low-density lipoprotein to evaluate coronary risk. Circulation. 2001 Apr 17;103(15):1930-2.
71. Holvoet P, Mertens A, Verhamme P, Bogaerts K, Beyens G, Verhaeghe R, et al. Circulating oxidized LDL is a useful marker for identifying patients with coronary artery disease. Arterioscl Throm Vas. 2001 May;21(5):844-8.
72. Ehara S, Ueda M, Naruko T, Itagane H, Haze K, Itabe H, et al. Plasma levels of oxidized low density lipoprotein (Ox-LDL) directly relate to the severity of the acute coronary syndromes. Circulation. 1998 Oct 27;98(17):765-.
73. Xu Y, Whitmer K. C-reactive protein and cardiovascular disease in people with diabetes: high-sensitivity CRP testing can help assess risk for future cardiovascular disease events in this population. Am J Nurs. 2006 Aug;106(8):66-72.
74. Nozue T, Fukui K, Yamamoto S, Kunishima T, Umezawa S, Onishi Y, et al. C-Reactive Protein and Future Cardiovascular Events in Statin-Treated Patients with Angina Pectoris: The Extended TRUTH Study. Journal of Atherosclerosis and Thrombosis. 2013;20(9):717-25.
75. Ferreiros ER, Boissonnet CP, Pizarro R, Merletti PFG, Corrado G, Cagide A, et al. Independent prognostic value of elevated C-reactive protein in unstable angina. Circulation. 1999 Nov 9;100(19):1958-63.
76. Danesh J, Wheeler JG, Hirschfield GM, Eda S, Eiriksdottir G, Rumley A, et al. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. N Engl J Med. 2004 Apr 1;350(14):1387-97.

77. Win HK, Chang SM, Raizner M, Shah G, Al Basky F, Desai U, et al. Percent change in B-type natriuretic peptide levels during treadmill exercise as a screening test for exercise-induced myocardial ischemia. Am Heart J. 2005 Oct;150(4):695-700.
78. Mueller C, Staub D, Zellweger M, Jonas N, Pfisterer M, Perruchoud A. Use of B-type natriuretic peptide in the detection of myocardial ischaemia. Eur Heart J. 2005 Sep;26:41-.
79. Paraskevaidis IA, Tsougos E, Varounis C, Dagres N, Karatzas D, Parissis J, et al. Exercise-induced changes of B-type natriuretic peptide uncover the unknown coronary artery disease in patients with chest pain and normal left ventricular systolic function. Eur J Cardiov Prev R. 2011 Feb;18(1):72-8.
80. Davidson NC, Pringle SD, Pringle TH, McNeill GP, Struthers AD. Right coronary artery stenosis is associated with impaired cardiac endocrine function during exercise. Eur Heart J. 1997 Nov;18(11):1749-54.
81. Shave R, Baggish A, George K, Wood M, Scharhag J, Whyte G, et al. Exercise-induced cardiac troponin elevation: evidence, mechanisms, and implications. J Am Coll Cardiol. 2010 Jul 13;56(3):169-76.
82. Yanowitz FG. Exercise ECG testing: Performing the test and interpreting the ECG results. Wwwuptodatecom.
83. Noman A, Ang DS, Ogston S, Lang CC, Struthers AD. Effect of high dose allopurinol on exercise in patients with chronic stable angina: A randomised, placebo controlled, crossover trial. Lancet. 2010;375:2161-7.
84. Tardif JC, Ford I, Tendera M, Bourassa MG, Fox K, Investigators I. Efficacy of ivabradine, a new selective I(f) inhibitor, compared with atenolol in patients with chronic stable angina. Eur Heart J. 2005 Dec;26(23):2529-36.
85. Tardif JC, Ponikowski P, Kahan T, Investigators AS. Efficacy of the I(f) current inhibitor ivabradine in patients with chronic stable angina receiving beta-blocker therapy: a 4-month, randomized, placebo-controlled trial. Eur Heart J. 2009 Mar;30(5):540-8.
86. Chaitman BR, Pepine CJ, Parker JO, Skopal J, Chumakova G, Kuch J, et al. Effects of ranolazine with atenolol, amlodipine, or diltiazem on exercise tolerance and angina frequency in patients with severe chronic angina: a randomized controlled trial. JAMA. 2004 Jan 21;291(3):309-16.
87. ; Available from: http://support.sas.com/documentation/cdl/en/statug/63962/HTML/default/viewer.htm#statug_mixed_sect.
88. National Institute for Health and Clinical Excellence (2011) Stable Angina (CG126). London: National Institute for Health and Clinical Excellence.
89. Kim J, Al-Mallah M, Juraschek SP, Brawner C, Keteyian SJ, Nasir K, et al. The association of clinical indication for exercise stress testing with all-cause mortality: the FIT Project. Archives of medical science : AMS. 2016 Apr 01;12(2):303-9.
90. Nielsen LH, Olsen J, Markenvard J, Jensen JM, Norgaard BL. Effects on costs of frontline diagnostic evaluation in patients suspected of angina: coronary computed tomography angiography vs. conventional ischaemia testing. Eur Heart J Cardiovasc Imaging. 2013 May;14(5):449-55.
91. Sekhri N, Feder GS, Junghans C, Eldridge S, Umaipalan A, Madhu R, et al. Incremental prognostic value of the exercise electrocardiogram in the initial assessment of patients with suspected angina: cohort study. BMJ. 2008 Nov 13;337:a2240.
92. Daly CA, Clemens F, Sendon JL, Tavazzi L, Boersma E, Danchin N, et al. The clinical characteristics and investigations planned in patients with stable angina presenting to cardiologists in Europe: from the Euro Heart Survey of Stable Angina. Eur Heart J. 2005 May;26(10):996-1010.

93. Min JK, Gilmore A, Jones EC, Berman DS, Stuijfzand WJ, Shaw LJ, et al. Cost-effectiveness of diagnostic evaluation strategies for individuals with stable chest pain syndrome and suspected coronary artery disease. Clin Imaging. 2017 May - Jun;43:97-105.
94. Fearon WF, Gauri AJ, Myers J, Raxwal VK, Atwood JE, Froelicher VF. A comparison of treadmill scores to diagnose coronary artery disease. Clin Cardiol. 2002 Mar;25(3):117-22.
95. Attar A, Mehrzadeh A, Foulad M, Aldavood D, Fallahzadeh MA, Assadian Rad M, et al. Accuracy of exercise tolerance test in the diagnosis of coronary artery disease in patients with left dominant coronary circulation. Indian Heart J. 2017 Sep - Oct;69(5):624-7.
96. Alexander KP, Shaw LJ, Shaw LK, Delong ER, Mark DB, Peterson ED. Value of exercise treadmill testing in women. J Am Coll Cardiol. 1998 Nov 15;32(6):1657-64.
97. Danad I, Szymonifka J, Twisk JWR, Norgaard BL, Zarins CK, Knaapen P, et al. Diagnostic performance of cardiac imaging methods to diagnose ischaemia-causing coronary artery disease when directly compared with fractional flow reserve as a reference standard: a meta-analysis. Eur Heart J. 2017 Apr 01;38(13):991-8.
98. Ebersberger U, Makowski MR, Schoepf UJ, Platz U, Schmidtler F, Rose J, et al. Magnetic resonance myocardial perfusion imaging at 3.0 Tesla for the identification of myocardial ischaemia: comparison with coronary catheter angiography and fractional flow reserve measurements. Eur Heart J Cardiovasc Imaging. 2013 Dec;14(12):1174-80.
99. Chaitman BR, Skettino SL, Parker JO, Hanley P, Meluzin J, Kuch J, et al. Anti-ischemic effects and long-term survival during ranolazine monotherapy in patients with chronic severe angina. J Am Coll Cardiol. 2004 Apr 21;43(8):1375-82.
100. Timmis AD, Chaitman BR, Crager M. Effects of ranolazine on exercise tolerance and HbA1c in patients with chronic angina and diabetes. Eur Heart J. 2006 Jan;27(1):42-8.
101. Tardif JC, Ponikowski P, Kahan T, Investigators A. Effects of ivabradine in patients with stable angina receiving beta-blockers according to baseline heart rate: an analysis of the ASSOCIATE study. Int J Cardiol. 2013 Sep 30;168(2):789-94.
102. Gomes-Neto M, Duraes AR, Reis H, Neves VR, Martinez BP, Carvalho VO. High-intensity interval training versus moderate-intensity continuous training on exercise capacity and quality of life in patients with coronary artery disease: A systematic review and meta-analysis. Eur J Prev Cardiol. 2017 Nov;24(16):1696-707.
103. Gremeaux M, Hannequin A, Laurent Y, Laroche D, Casillas JM, Gremeaux V. Usefulness of the 6-minute walk test and the 200-metre fast walk test to individualize high intensity interval and continuous exercise training in coronary artery disease patients after acute coronary syndrome: a pilot controlled clinical study. Clin Rehabil. 2011 Sep;25(9):844-55.
104. Jaureguizar KV, Vicente-Campos D, Bautista LR, de la Pena CH, Gomez MJ, Rueda MJ, et al. Effect of High-Intensity Interval Versus Continuous Exercise Training on Functional Capacity and Quality of Life in Patients With Coronary Artery Disease: A RANDOMIZED CLINICAL TRIAL. J Cardiopulm Rehabil Prev. 2016 Mar-Apr;36(2):96-105.
105. Rognmo O, Moholdt T, Bakken H, Hole T, Molstad P, Myhr NE, et al. Cardiovascular risk of high- versus moderate-intensity aerobic exercise in coronary heart disease patients. Circulation. 2012 Sep 18;126(12):1436-40.
106. O'Donovan G, Owen A, Bird SR, Kearney EM, Nevill AM, Jones DW, et al. Changes in cardiorespiratory fitness and coronary heart disease risk factors following 24 wk of moderate- or high-intensity exercise of equal energy cost. J Appl Physiol (1985). 2005 May;98(5):1619-25.